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NOTES ON THE BACTERIOLOGICAL EXAMINATION OF THE SOIL OF PHILADELPHIA.

NOTES ON THE BACTERIOLOGICAL EXAMINATION OF THE SOIL OF PHILADELPHIA.

By MAZYCK P. RAVENEL, M. D.

Scott Fellow in Hygiene, 1893-94.

Presented by Dr. J. S. BILLINGS.

This work was undertaken at the instance of Dr. John S. Billings, Director of the Laboratory of Hygiene, University of Pennsylvania, with the view of obtaining, if possible, some explanation, from a bacteriological standpoint, of the greater mortality among persons living in houses built on made ground over those living on virgin soil. In some cities, as Washington and New York, filled localities can be mapped out and the course of old runs followed by the higher death rate in these districts. No positive opinion can be based on the data so far obtained, and these notes are presented mainly as a small contribution to our knowledge of the bacteria of the soil.

In the prosecution of the work I have had the advice of Dr. Billings and of Dr. A. C. Abbott, first assistant, to both of whom I am much indebted.

SUMMARY.

The work has extended over a period of ten months, with considerable interruption during much of the time.

Virgin soil was examined to a depth of 12 feet, samples having been obtained from the walls of an excavation at intervals of a foot. Samples of made soil were obtained in the same way to a depth of 9 feet. Other samples of both made and virgin soil were obtained by Fränkel's earth borer to a depth of 5½ feet.

From these samples of earth 71 cultures were isolated and carefully studied. Wherever possible they were identified with already described species, and with each other, the final result being 36 varieties of organisms, of which 29 are believed to be new.

Morphology.—The bacillus forms predominate largely. Only two cocci were found, both new. No spirilla were found, and but one sarcina. Five varieties of cladothrix were found, only one of which has been identified with already described species—the *Cladothrix dichotoma* of Cohn. This species has been found repeatedly at different depths in both made and virgin soils, being apparently the most common and widely distributed form of the genus.

Depth.—The greatest depth at which bacteria have been found in virgin soil was 6 feet, at which depth the bacillus Megatherium and the bacillus ramosus were observed. All samples of virgin soil below this were found to be sterile.

This result agrees closely with the experiments of Koch and Fränkel. The former in 1881 found that few bacteria were to be found 1 meter below the surface in soil which had not been disturbed. Fränkel found that the number of organisms decreased greatly and suddenly at a depth of three-fourths of a meter to 1½ meters, while at the latter depth life was absent in many instances. He obtained, however, in other experiments, colonies from a depth of 3 and 4 meters, which took a long time to make their appearance (Sternberg). Only one of the varieties most commonly found by Fränkel in the deeper layers of the soil was encountered in these experiments, the bacillus ramosus. In two experiments a sterile layer was found at the depth of about 1 meter, below which a considerable number of bacteria were observed.

From made soil, although many years old and paved over, numerous colonies were obtained from a depth of 9 feet. No actual count was made, but the number of bacteria at this depth was apparently as great as at the surface.

Method of collecting samples.—Whenever possible, the samples were taken from the wall of a freshly made excavation, the wall being dug away to a depth of several inches just before taking the sample, and care was exercised that the pick should not touch the spot from which the earth was taken. A short tube of thick glass, previously plugged and sterilized, was bored into the fresh surface until a sufficient quantity was obtained. In all other instances the earth borer of Fränkel was used, but was not found satisfactory, besides being difficult to operate, the soil being a very stiff clay containing numerous pebbles.

Method of examination of samples.—Two methods of examination have been suggested. In one—that preferred by Fränkel in making counts—a portion of the earth to be examined is put into liquefied gelatin and thoroughly broken up with a sterile rod, after which it is mixed by tilting the tube up and down, revolving it at the same time, and lastly rolled. The second method consists in crushing the earth in a tube of sterile water and washing it well by agitation. A certain amount of the water is then introduced into the liquid medium and roll tubes made. Both methods were tried, the former being found to be more satisfactory. It was used exclusively, with the modification of pouring the gelatin into Petri dishes instead of making roll tubes; and in every instance dilutions were made from the original tube, in order to better isolate the colonies. To determine the temperature at which the cultures should be kept, duplicate plates on agar-agar and gelatin were made from several samples of earth, following the second method described. After washing the earth thoroughly in sterile water, equal quantities of the water were mixed into tubes of liquid agar-agar and gelatin and then poured into Petri dishes. All of the gelatin and a duplicate series of the agar-agar plates were kept at room temperature, the others being placed in the incubator at 35° to 36° C. At the end of thirty-six hours a count showed that those plates kept at room temperature contained the greater number of colonies. In all the subsequent experiments gelatin has been used and the plates kept at room temperature. The wisdom of this has been confirmed by the subsequent study of the individual organisms isolated, since quite a large proportion of them grow better at room temperature than at 35° to 36° C., and some will not grow at all at the latter temperature.

Study of organisms.—The bacteria isolated have each been studied as to their morphology, both in stained preparations and in the hanging drop; as to motility and cultural peculiarities; all have been stained for flagella after the method of Löffler, and all have been placed in an atmosphere of pure hydrogen and the growth noted. The culture media employed have been those given in the text-books, and the percentages have not been changed except in very hot weather, when it was found necessary to add gelatin in the proportion of 12 per cent instead of the 10 per cent used during most of the work.

CONCLUSIONS.

As stated at the outset, the data gained during this study are not sufficient to base any positive conclusions upon. The following propositions have been substantiated to a greater or less extent:

(1) Made soils, as commonly found, are rich in organic matter, and excessively damp through poor drainage.

(2) They furnish conditions more suited to the multiplication of bacteria than do virgin soils, unless the latter are contaminated by sewage or offal.

(3) Made soils contain a larger number of bacteria per gram, and the number of species is greater, while the deeper layers are as rich, both in numbers and varieties, as are the upper ones. After some years the number in the deeper layers probably becomes proportionately less.

(4) There is a greater probability that made soils may contain pathogenic bacteria.

THE CULTIVATION OF ANAËROBIC BACTERIA.

Among the organisms which find their habitat in the upper layers of the soil, several of the most interesting and important, from a pathological point of view, belong to the class of strict

anaërobes. Their study presents considerable technical difficulties. Of the various methods proposed for the cultivation of these bacteria, two appear to be of special value. These are the method of Buchner (Fig. 1), in which the oxygen is absorbed by means of pyrogallic acid, leaving a residual atmosphere of nitrogen; and, second, the employment of an atmosphere of hydrogen. Buchner's method has the objectionable feature of causing a negative pressure, which puts the bacteria under unnatural conditions. All things considered, the use of an atmosphere of hydrogen seems the most practical method we possess, and is favorable to the growth of the most important anaërobic organisms known to us.

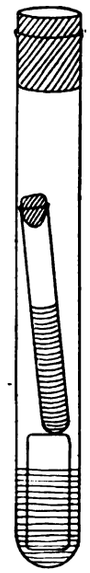


FIG. 1.

For plate cultivation, an apparatus similar to that described by Liborius in the Zeitschrift für Hygiene, Vol. I, page 128, is most useful. It consists of a bell jar having openings at the top and near the bottom, in which perforated rubber stoppers, bearing glass tubes with stopcocks, are inserted. The jar and plate on which it rests are put in a frame by which they can be firmly clamped together. A ring of soft rubber is put between the jar and plate, and it is well to supplement this by several coats of melted paraffin applied outside. The air is pumped out from the bottom and hydrogen introduced at the top, the process being repeated three times, at least, in order to insure the removal of all oxygen. It is well to put a vessel containing about 50 c. c. of an alkaline solution of pyrogallic acid under the jar also, as it shows whether the apparatus is leaking or not, and at the same time serves to absorb any oxygen which may have remained. (See Fig. 2.)

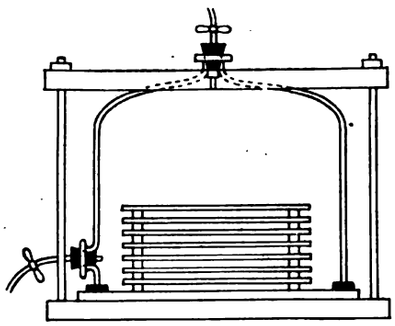


FIG. 2.

For test-tube cultures the method of Fränkel (Fig. 3), or, better, the method of Sternberg (Fig. 4) may be employed. In the former an ordinary tube is closed by a soft-rubber stopper, through which two glass tubes pass, one reaching nearly to the bottom of the test tube, for the introduction of the hydrogen; and the other, a short one, for the escape of the gas. The method of Sternberg is essentially a modification of this. After inoculating the tubes the cotton plug, or a part of it, is pushed down into the neck of the tube. Above this a section of a rubber stopper, carrying two glass tubes, both short, is inserted for about a half inch. The space above is coated with melted sealing wax, which does not contract on cooling. The tube is then attached to the hydrogen generator and inverted, the gelatin or agar being solidified. The hydrogen being light rises into the tube, displacing the air. After a sufficient time the outlet tube is sealed in the flame, then the inlet also. Both of these methods are useful mainly for the study of colonies in roll tubes, and the latter can be used only for solid cultures. The employment of the air pump attached to the outlet tube facilitates matters greatly and enables Sternberg's method to be applied to liquid cultures. Should Sternberg's method be used the tubes should be freshly sterilized and the top of the plug of cotton burned off, as otherwise the culture will soon be contaminated with moulds in abundance, many of which thrive in an atmosphere of hydrogen.

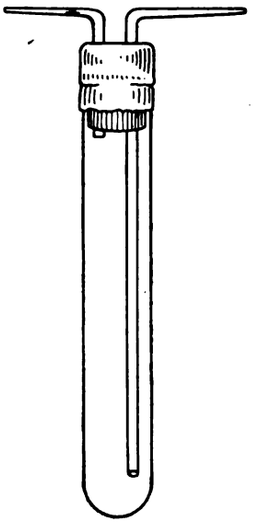


FIG. 3.

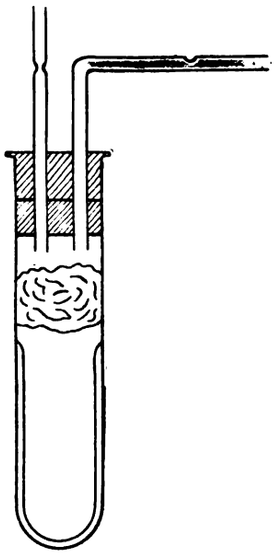


FIG. 4.

The most simple method of test-tube culture is the employment of a jar or bottle, in which a number of tubes may be placed at the same time. The apparatus of Liborius above described

may be used for this purpose, or an ordinary wide-mouthed bottle, fitted with a rubber stopper carrying two tubes, one reaching to the bottom, the other extending only through the stopper, serves the purpose well. In the Centralblatt für Bakteriologie und Parasitenkunde, for November, 1893, Dr. F. G. Novy describes a jar devised by him which embodies this principle, and has been found very satisfactory by him (Fig. 5). Using the same principle, I have designed a jar for what may aptly be termed cultivation "en masse," which has advantages not possessed by any other apparatus for the same purpose with which I am acquainted. It fulfills every requirement perfectly, if well constructed, and is easy to operate. The general plan is modeled after the jar used for drying gases, known as "Fresenius's Improved" calcium chloride jar. It may be purchased of any dealer in laboratory supplies, and even without modification is very useful. The older forms may also be used by fitting rubber corks carrying glass tubes to the mouth and to the lower opening.

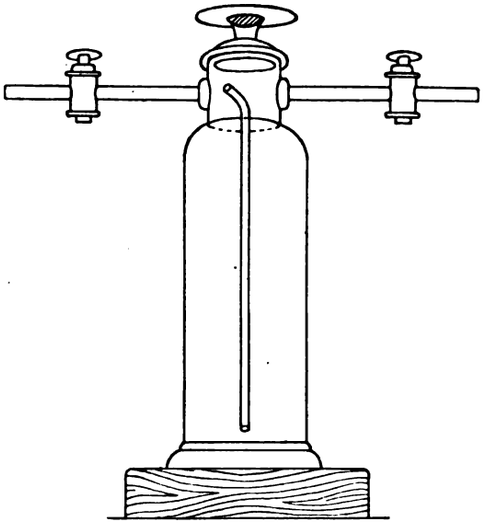


FIG. 5.

As adapted to the cultivation of anaërobic bacteria, the jar is as follows: It consists essentially of a glass cylinder, with a good base, and having some 2 inches from the bottom a constriction, dividing it into two chambers which communicate. Opening into the bottom chamber, at the shoulder, is a corrugated glass tube, fitted with a well-ground stopcock. The mouth of the jar is large, and fitted with a perforated glass stopper, the perforation opening into the cavity of the jar. A corrugated glass tube is attached to the neck of the jar, through which a hole has been made continuous with the perforation of the stopper. By turning the stopper this is closed, sealing the jar at the top. (See Fig. 6.) The jar may be made of different sizes, the following dimensions being most serviceable for general use: Diameter of upper chamber, 3 inches; of mouth (inside), 1.5 inches; height of upper chamber (inside, clear of shoulders), 7 inches; diameter of lower chamber, 3 inches; height of lower chamber, 2 inches.

A most essential feature is that the stopper and the stopcock shall be accurately ground, so as to be absolutely air-tight. The height of the upper chamber is adapted to the length of the test tubes one wishes to use, and the diameter to the number of cultures most convenient. The use of the apparatus is as follows: A piece of wire gauze is inserted, which rests on the shoulders of the constriction, and completes the floor of the upper chamber. On this a layer of cotton is placed. All the media to be used are steamed for fifteen to twenty minutes, and cooled rapidly in ice water. The tubes having been inoculated are then introduced and the jar closed. Into the lower chamber is introduced, through the lower tube, an alkaline solution of pyrogallic acid, enough being put in to fill the chamber up to the opening of the tube. The lower tube is then at once connected with the air pump, and the upper one with a hydrogen generator, the stopper being so turned as to close the jar. When the air has been exhausted, as indicated by the vacuum meter, the lower stopcock is closed and the jar filled with hydrogen. The hydrogen is then cut off and the jar again exhausted, and again filled with hydrogen, the process being repeated three times in order to make sure of all the oxygen being removed. The jar is then sealed above and below, and may be put in the incubator if no gelatin has been used.

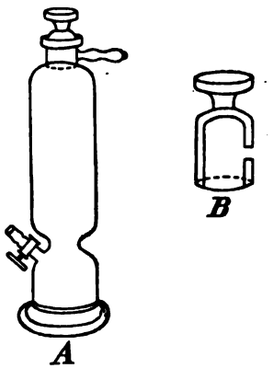


FIG. 6.—*A*, jar; *B*, sectional view of stopper.

The strength of the solution of pyrogallic acid used is one-half that recommended by Buchner for his method, viz, 1 gram of pyrogallic acid to 20 c. c. of a 10 per cent solution of caustic potash. The use of the pyrogallic acid is not necessary if the jars be well constructed, but is employed as a safeguard, and is useful as an indication of the efficiency of the apparatus.

The most convenient tube for use in this jar is 6 inches long by three-eighths of an inch in diameter.

A word may be said about hydrogen generators. The apparatus of Kipp has a wide use, but has not proved altogether satisfactory where a considerable quantity of the gas is desired. The apparatus of Sternberg is an excellent one, and simple in construction (Fig. 7). (Manual of Bacteriology, p. 83.) I have found much satisfaction in the use of an apparatus constructed as follows (Fig. 8): Two bottles holding from 3 to 4 liters are fitted with perforated rubber stoppers, that of bottle *A* carrying two tubes, one reaching to the bottom of the bottle, while the other is short, bent at right angles, and fitted with a stopcock. The stopper of bottle *B* also carries a tube reaching to the bottom, and into the second hole a glass plug is placed, for which a safety tube may be substituted. By the use of the plug the gas can be stored in greater quantity under some pressure. Broken glass is put into bottle *A* for a depth of about an inch, and on this the zinc is placed. The long tubes are connected with stout rubber tubing, on which it is well to have a screw pinchcock. Bottle *B* is raised some 4 inches above *A*, and the dilute acid placed in it. To start the apparatus, slight suction is applied to the outlet tube of bottle *A*. As soon as the acid starts to flow it will go by the action of the siphon into bottle *A*. When enough has gone over, the pinchcock is closed to prevent the suction of air. On closing the stopcock of the outlet tube the acid is forced back into the upper bottle, and the zinc is left dry. By using a stopper in the upper bottle instead of a safety tube, there is always enough pressure to force the acid over into *A*, making the apparatus practically automatic. As it is almost always used in connection with an air pump, however, this is not a matter of importance. The stopcock of the outlet should never be turned off when the tube connecting the two bottles is closed, otherwise an explosion is apt to result. With this apparatus one has always at hand from 3 to 4 liters of hydrogen under pressure, and it is a simple matter to fill any vessel, from a test tube to a bell jar. In every case the air is first exhausted, and then the hydrogen let in, the process being repeated three or more times.[1]

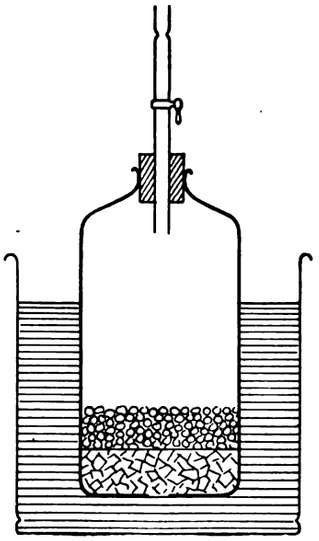


FIG. 7.

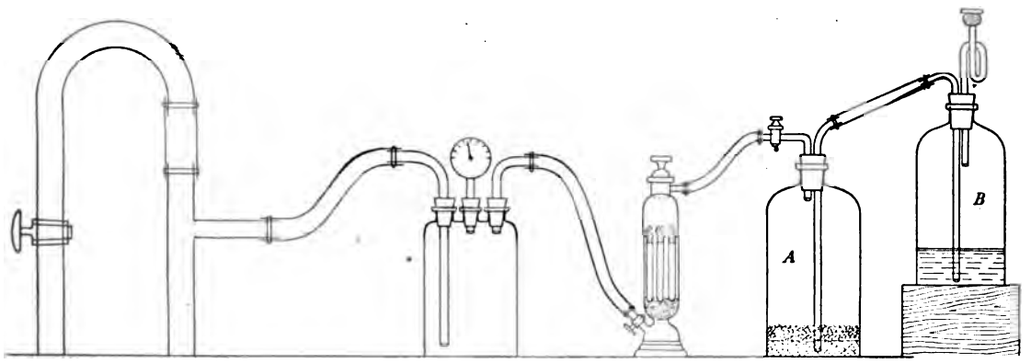


FIG. 8.

For the purpose of exhausting the air, the most convenient pump is the simple one constructed on the principle of Sprengel's mercurial pump, in which a stream of water does the work. It is attached to a spigot, and the suction tube is connected with a Woulf bottle to prevent any back flow of water.

The entire apparatus, including the anaërobic jar, is shown in Fig. 8.

[1] This apparatus was suggested to me by Dr. S. S. Kneass, a former student in Professor Roux's laboratory at the Pasteur Institute, where he saw a similar one in use.

DESCRIPTION OF ORGANISMS ISOLATED.

MICROCOCCUS ORBICULARIS FLAVUS.

Found in virgin soil at the depth of 5 feet.

Character.—Shows slight growth in an atmosphere of hydrogen.

Morphology.—Large cocci, irregularly grouped.

Spore formation not observed.

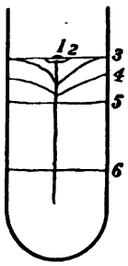
Motility.—Non-motile.

Flagella not demonstrated.

Colonies on gelatin plates.—The colonies first make their appearance about the third day, as minute, pale yellowish dots. ×80. No distinction can be made between the deep and surface colonies, except that the latter are larger. They appear as homogeneous, or, sometimes, slightly granular, disks, with even edges and a yellowish hue. The growth is very slow, and at the end of five days the surface colonies are less than 1 mm. in diameter, round, even edged, and only slightly elevated. ×80. They show a homogeneous center, with a finely granular margin. The edges are even and clean-cut. The deep colonies are rather irregular in shape, yellow in color, and finely granular. Here and there they appear to be folded. There is nothing distinctive about either the deep or the surface colonies. The colonies lie in a saucer of liquefied gelatin, which is circular and has smooth margins to the naked eye.

Agar slant.—A faint yellowish line forms by the second day, which increases rather slowly. At the end of a week a canary-colored layer, some 2 mm. wide, has formed. It has a smooth, glistening surface and rather uneven margins.

Gelatin stab.—Growth occurs deep down the puncture, and a small yellow button forms on the surface, which is soon floating in a saucer of liquefaction. Liquefaction is rather slow, being complete in four to five weeks. The floor becomes level at the end of a week, and is covered with a yellowish flocculent deposit, while the liquefied gelatin is cloudy.



Gelatin stab.

Potato.—A moist, colorless, very thin layer forms over a large part of the surface by the third day. It becomes thicker and turns yellow about the fourth day. It then becomes somewhat granular looking, and is moist and shining, the color being somewhat deeper than on agar.

Bouillon.—Becomes diffusely cloudy by the second day. A white deposit forms on the bottom, which becomes a faint yellow after some time.

Rosolic acid.—Becomes slightly darker after two weeks.

Litmus milk.—No change of any kind can be seen. Reaction amphoteric.

Sugar gelatin, deep stab.—Growth quite deep along the puncture. Liquefaction is more rapid than in plain. No gas formed.

Indol.—Faint reaction with both sulphuric acid and sodium nitrite.

Relation to temperature.—Grows more rapidly at room temperature than in the incubator.

BACILLUS AURESCENS.

Found in virgin soil at the depth of 30 inches.

Character.—Strict aërobe. No growth in an atmosphere of hydrogen.

Morphology.—Short, straight rods with oval ends, making the cells spindle shaped. Length from two to three times as great as breadth. Found singly.

Spore formation not positively demonstrated. In some of the rods are seen oval, bright spots which resemble spores somewhat.

Motility very slight.

Flagella not demonstrated.

Colonies on gelatin plates.—Colonies seen at the end of thirty-six to forty hours as minute whitish points, which may easily escape observation. ×80. No distinction can be made out between the deep and surface colonies at this stage. They appear as coarsely granular disks of

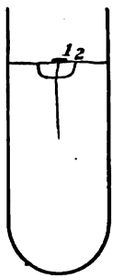
a brownish cast, and have even edges. At the end of four days the surface colonies are one-half of a millimeter in diameter and yellow. They seldom attain a greater size than this. The deep colonies are punctiform and whitish. ×80. Both deep and surface colonies have much the same structure and neither show any distinctive features, even after ten days. They are yellowish-brown granular disks with even margins. The surface colonies became orange yellow after some days. The gelatin may be softened, but no liquefaction was observed.

Agar slant.—A thin faintly yellow line forms by the second day, which has spread irregularly near the bottom. It increases rather slowly and becomes viscous or tenacious. The color becomes a golden yellow.

Gelatin slant.—Forms a yellow band, with even edges, and not much elevated. If the inoculating needle contains but little of the culture, isolated circular colonies about 1 mm. in diameter are formed along the line of inoculation.

Gelatin stab.—Slight development occurs along the upper part of the puncture, while a yellow button of growth forms on the surface, reaching a diameter of 1 mm. by the fourth day. At the end of ten days the top growth has become somewhat sunken, and in eighteen to twenty days a cup-shaped depression about 5 mm. deep has formed, but no liquefaction can be detected evaporation keeping pace with it.

Potato.—A moist yellow band forms along the line of inoculation in twenty-four hours. It soon spreads over much of the plug in a thick moist layer, and the color deepens to an orange yellow.



Gel. stab.

Bouillon.—Slight cloudiness is observed on the fourth day, the growth being slow. This increases later, and dense flocculi which sink to the bottom are formed.

Rosolic acid.—Very slight growth. Color becomes slightly deeper after eight to ten days.

Litmus milk.—Becomes lighter after a week, and the color is slowly discharged without turning red. There is no coagulation of the casein.

Sugar gelatin, deep stab.—Growth confined to the surface. No gas is produced.

Indol.—Reaction negative.

Relation to temperature.—Grows somewhat more rapidly at 35° to 36° C. than at room temperature.

Note.—This resembles the bacillus Flavescens (Pohl) very closely, as far as the published descriptions go. The former is non-liquefying, while this is a very slow liquefier. In fact, no liquefaction could be detected, but was inferred from the depression of the surface growth on gelatin.

Bacillus Fluorescens Ovalis.

Found at a depth of 2 feet in a cultivated field.

Character.—Facultative anaërobe.

Morphology.—Short straight rods, with rounded ends, from two to three times as long as broad. Occurs singly. Many of the rods show irregular staining.

Spore formation not positively demonstrated.

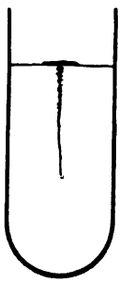
Motility.—Actively motile.

Flagella are polar.

Colonies on gelatin plates.—Colonies are seen at the end of twelve hours as minute translucent grayish dots. × 80. The deep are pale gray, finely granular disks, with even margins. The surface colonies are irregularly circular, pale gray, and finely granular, with even edges. At the end of three days the surface colonies are 1 mm. in diameter and a bluish white color; the deep are still punctiform. ×80. They are yellowish and almost homogeneous, some faint markings being seen near the center. At the end of eight days the surface colonies are 1 mm. in diameter, circular, with well defined margins, and greenish white in color. Deep are punctiform. ×80. Deep show no change, except in density and in color, which is now yellowish. Those on the surface are also more dense, and show little or no structure. Near the margins darker lines can be faintly seen radiating from the center. There is no liquefaction nor discoloration of the gelatin.

Agar slant.—A thin greenish white band forms along the line of inoculation by the second day, and the agar has acquired a faint green tint. It soon spreads, reaching the tube wall for the lower third, and has leafy margins. The agar becomes a beautiful yellowish green.

Gelatin stab.—Growth occurs for some distance down the puncture, and a white button with irregular leafy margins forms on the surface. After several days fine spinous outgrowths form along the puncture, about 1 mm. long and whitish in color. The gelatin becomes faintly green near the surface. There is no liquefaction.


Gel. stab.

Potato.—By the second day a very thin moist layer about the color of honey has formed over a large part of the surface. After some time this becomes a pale yellowish brown. It remains very thin and has no distinctive features.

Bouillon.—Becomes diffusely cloudy and an incomplete flaky pellicle forms on the surface. After five days the bouillon becomes a faint greenish tint, and is opaque, while a white mass forms at the bottom of the tube.

Rosolic acid.—Becomes slightly darker after five days.

Litmus milk.—After a week the color becomes more blue, and at the end of four weeks is a slaty blue. A whitish deposit forms on the bottom of the tube, but there seems to be no change in the milk itself. The reaction is decidedly alkaline.

Sugar gelatin, deep stab.—Growth quite deep along the puncture, and a button on the surface. There is no liquefaction nor gas production.

Indol.—Reaction negative.

Gelatin slant.—A greenish white band with beaded edges forms along the line of inoculation. The gelatin acquires a green tint about the third day. No liquefaction occurs.

Relation to temperature.—Grows well at both room temperature and at 35° to 36° C.

Sarcina Subflava.

Found at the surface of a cultivated field.

Character.—No growth in an atmosphere of hydrogen.

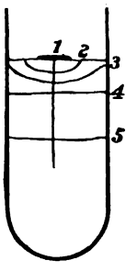
Morphology.—Packets square and longer than broad, showing 4, 8, 16, 32, or more elements on each face.

Spore formation not observed.

Motility.—Non-motile.

Flagella not demonstrated.

Colonies on gelatin plates.—Colonies become visible in thirty-six to forty hours as minute yellowish dots. ×80. They are finely granular, yellowish disks with even margins. By the fourth day the surface colonies are 1 mm. in diameter, a pale yellow, and are slightly sunken in the gelatin. ×80. They are a pale yellow, homogeneous, and have even margins. After some days the margins may become irregular and look more or less granular. The deep colonies increase very slowly and only change in appearance by becoming more dense. Liquefaction is rather slow. There is nothing distinctive about the colonies at all.



Gel. stab.

Agar slant.—A yellowish band, 3 mm. wide, forms by the fourth or fifth day. It is smooth, pale yellow, and has irregular beaded margins.

Gelatin stab.—Growth quite deep along the puncture, and a small almost white button forms on the surface, which has irregular margins. Slow liquefaction takes place under this in saucer shape. At the end of ten days the liquefaction is 1 cm. deep and has a level floor. The liquid is cloudy.

Potato.—No growth could be obtained after a number of trials.

Bouillon.—Whitish flocculi form at the bottom, and after several days a thin pellicle forms on the surface. The bouillon does not become clouded.

Rosolic acid.—No change observed.

Litmus milk.—No change either in color or in the milk itself after four weeks. Reaction faintly acid.

Sugar gelatin, deep stab.—Growth quite deep and on the surface, with slow liquefaction. No gas production.

Fig. 1.

Bac. Megatherium.
Surface colony 18 hrs. old. x 80.

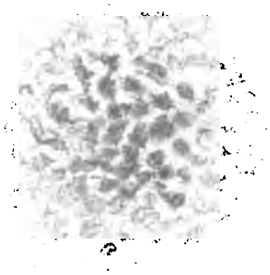
Fig. 2.

Bac. Megatherium.
Colony which has just reached the surface.
36 hrs. x 80.

Fig. 3.

Bac. Megatherium.
Surface colony 60 hrs. old. x 80.

Fig. 4.



B.

Fig. 5.

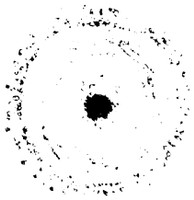
A.

Bac. Megatherium.
Forms of surface colonies.
A. 2 days old. B. 3 days old. x 80.

Fig. 6.

Bac. Formosus.
Surface colony 60 hrs. old. x 80.

Fig. 7.



Bac. Formosus.
Surface colony on 7th day. x 80.

Fig. 8.

Fig. 9.

A

B.

Bac. Verticellatus.
A. Deep colony 14 hrs. old. x 80.
B. Surface colony 14 hrs. old. x 80.

Fig. 10.

Cladothrix Dichotoma.
Deep colony 24 hrs. old
Surface colonies exactly the same, only larger. x 80.

A. HOEN & CO., LITH., BALTIMORE.

Indol.—Reaction negative.

Relation to temperature.—Grows well at both room temperature and at 35° to 36° C.

Note.—This may be identical with the *Sarcina flava* (De Barry). The published descriptions are so meager that positive identification has not been possible. The chief difference noted has been in the growth on potato. I have not been able to obtain any growth, while the growth of the *Sarcina flava* is scanty and limited to the line of inoculation.

Bacillus Megatherium.

(Figs. 1, 2, 3, 4, and 5, Pl. I.)

Very common in soil, both made and virgin, at all depths where bacteria are found at all. In the course of this work it was found as low as 7 feet in made soil which had been paved for a number of years.

Character.—It is a strict aërobe, no development occurring in an atmosphere of hydrogen.

Morphology.—Thick straight rods, with rounded ends, from three to five times as long as broad. It forms quite long chains, in which the rods are often bent on each other and are of unequal lengths. The rods show a peculiar granulation of their contents, said to be peculiar to this organism.

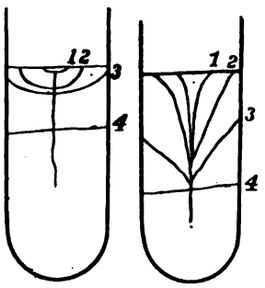
Spore formation is usually well advanced at the end of sixteen hours in a warm room. The rods appear to grow shorter and oval, while the center is occupied by a long oval spore almost as long as the rod itself. In cultures thirty-six hours old chains of spores are seen, which are often pushed together, the ends overlapping each other.

Motility.—Slight movements are seen, which have been described as "amœboid."

Flagella not demonstrated.

Colonies on gelatin plates.—Colonies are seen at the end of twelve to sixteen hours as whitish dots. ×80. The deep appear brown and have regular margins. No structure can be made out. Those on the surface are lighter in color and have very irregular margins. The center is too dense to show much structure, but where any can be made out consists of a cloudy mass of interwoven and tangled filaments. By the third day the surface colonies are each in a saucer of liquefaction, 4 mm. in diameter, in which floats a whitish island with irregular margins, surrounded by a zone of opaque grayish liquefied gelatin. ×80. Deep are unchanged. The surface show the dense cloudy masses in the center, merging into the lighter masses made up of broken and tangled filaments floating in the liquefied gelatin. The margins of the saucer of liquefaction are even. When a colony has begun beneath the surface and broken through there is a well-defined darker center, surrounded by a ragged fringe of coarse filaments. In some colonies this fringe is much more regular, and assumes a festoon-like arrangement, going out in bundles at tolerably regular intervals, which divide to the right and left, joining those next them, thus forming a series of loops or festoons around the colony. This arrangement is lost in twenty-four hours by the liquefaction of the gelatin, and only broken filaments can be seen around the colony.

Agar slant.—The growth is at first a creamy white color, and forms abundantly, sometimes being piled up 1 mm. in height. The surface is moist, smooth, and glistening. It often becomes yellowish in color, and forms a thick pasty mass, with a strong smell of stale milk. After several generations on agar it changes its character, apparently, and forms a thin dirty gray layer, the agar becoming brownish after a time.



Gelatin stab.

Gelatin stab.—Liquefaction occurs in two different ways, according to the vigor of the growth apparently. In the first a saucer of liquefaction is formed, which increases, reaching the tube wall and becoming deeper. Whitish flocculi float in the liquid and gradually settle to the floor, which becomes level. In other cultures growth takes place deep down the stab, and liquefaction occurs in funnel form by the second day. The floor becomes level after ten to twelve days.

Potato.—A thick band, whitish and looking like melted candy, is formed by the second day. It is smooth and glistening. It increases rapidly, and is thrown into large folds and wrinkles by

the third or fourth day, and becomes cream colored. The folds soften down, or are overgrown in a few days, and the layer becomes a shiny looking dirty cream or putty color. It has a strong musty odor, like stale milk.

Bouillon.—Becomes diffusely clouded in thirty-six hours, and has large white flocculi floating in it. These settle to the bottom, forming a dense whitish deposit, while the bouillon remains almost entirely clear.

Litmus milk.—The milk becomes stratified, being lightest at the bottom, and somewhat reddish at the top, where it is watery and translucent. The color is discharged slowly and the casein apparently dissolved. Reaction neutral. In some cultures the milk becomes more blue, and after a time, a beautiful violet color by transmitted light, with a decidedly alkaline reaction. the casein being dissolved.

Sugar gelatin, deep stab.—Growth the same as in plain, but liquefaction takes place much more rapidly. No gas is produced.

Rosolic acid.—No change in color is noticed.

Indol.—Reaction negative.

Relation to temperature.—Grows somewhat more rapidly at 35° to 36° C.

Note.—The above description differs from those found in most books in some respects. I have found considerable differences in different cultures of *Megatherium*, and cultures recently obtained from the earth behave differently than when kept on artificial culture media for some time. As Fränkel says, "It seems almost as if a continued nutrition with our usual food media did not agree with this mocro-organism." And speaking of the appearance of the rods he says, * * * "and one might be tempted to suppose that a new variety had arisen, were it not always easy to breed normal cells again from these monstrous and crippled forms by employing a more suitable culture medium." (Text-book of Bacteriology, American edition.)

I have found this true as regards the cells themselves, but the growth in the different media is never quite the same as when the cultures are fresh from the soil, so much so that it is often hard to believe that one has the same organism.

Bacillus Formosus.

(Figs. 6 and 7, Pl. I.)

Found in made soil a number of years old.

Character.—Requires oxygen for good development, but shows some growth in an atmosphere of hydrogen.

Morphology.—Slender, straight rods, with rounded ends, from seven to eleven times as long as broad. Occurs singly and in twos and threes.

Motility.—Slight independent movements noticed.

Flagella not demonstrated.

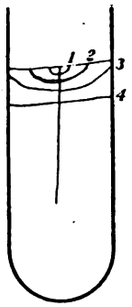
Spore formation doubtful. Probably does not form spores.

Colonies on gelatin plates.—Colonies are seen in twenty-four to twenty-eight hours as minute greenish-white dots. At the end of thirty-six hours the deep are still punctiform, while those on the surface are one-fourth of a millimeter in diameter. × 80. The deep are yellowish disks, with even edges and finely granular contents. The surfaces are circular, have even edges, and are yellowish in the center, fading to gray at the edges. They are finely granular, with no distinctive features. At the end of sixty hours the deep colonies are yellowish dots, while on the surface they are white, and one-half of a millimeter in diameter. × 80. Deep show no change. On the surface the colonies are made up of a nucleus, more or less distinct in the different colonies and yellowish brown in color. Around this is a zone of lighter yellowish hue, traversed by brownish wavy lines. Beyond is a zone of much darker color, and fading into pale yellow at the margins. It is made up of lines radially disposed, with here and there cloudy masses of brownish granules. The edges are regular and smooth. At the end of a week the surface colonies are about 1 mm. in diameter. × 80. Many still show a nucleus, and the zones above described. In the majority the nucleus and zone next to it have become of the same color, and merge into a dark-brown zone, which fades into a lighter marginal zone. In all the colonies, numbers of amorphous

granules are seen scattered through the colony without any attempt at arrangement. The edges have lost their distinct outline, and the granules are seen invading the gelatin beyond. Rather slow liquefaction takes place.

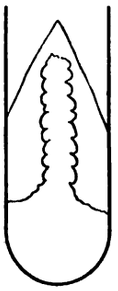
Agar slant.—A whitish band, 1 mm. wide, with notched edges forms in twenty-four hours. By the third day this has become 4 mm. wide and spread to the tube wall near the bottom. It is a smooth, white, glistening band with notched edges, and not very thick.

Gelatin stab.—By the third day a small saucer of liquefaction has formed, 2 mm. in diameter, while the growth along the puncture is very scanty. Liquefaction is slow, and by the end of two weeks has reached a depth of 1 cm. The floor becomes level and is covered with white flocculi, while the liquefied gelatin is almost perfectly clear.



Gel. stab.

Potato.—A moist white band is formed along the line of inoculation. It spreads widely where moist, and becomes a cream color by the fourth day. Later it spreads all around the plug and changes to a dirty cream color, and is brownish at the edges where it is thin.


Agar slant.

Bouillon.—Diffuse cloudiness is caused in twenty-four hours; a dirty white deposit forms on the bottom of the tube.

Rosolic acid.—Becomes slightly darker.

Litmus milk.—Becomes more blue by the third day, after which the color is rapidly discharged, being gone by the tenth day. There is no coagulation of the casein. A white deposit collects on the bottom of the tube.

Sugar gelatin, deep stab.—Growth confined to upper part of puncture, with liquefaction in saucer shape. No gas is produced.

Indol.—Reaction negative.

Relation to temperature.—Grows better at room temperature than at 35° to 36° C.

Bacillus Verticillatus.

(Figs. 8 and 9, Pl. I.)

Found at a depth of 7 feet in made soil which had been paved over for a number of years.

Character.—Good growth in an atmosphere of hydrogen, with extensive liquefaction.

Morphology.—Thick, straight rods, with rounded ends, from three to five times as long as broad. Forms chains of ten to twelve elements.

Spore formation observed only in potato cultures after two weeks. They are oval and formed in the center of the rod.

Motility.—Doubtful; slight, if at all.

Flagella not demonstrated.

Colonies on gelatin plates.—Colonies appear in twelve hours, and in fourteen hours those on the surface have attained a diameter of 1 mm., while the deep are one-fourth of a millimeter, and whitish. The surface colonies are each in a small saucer of liquefaction, with even circular margins. They are grayish in color and translucent. × 80. Deep are grayish looking, and consist of a tangled mass of fine filaments, surrounded by quite a deep fringe of fine lines arranged in a whorl from right to left. The surface colonies are filled with slightly opaque liquefied gelatin, in which fine tangled lines like a ball of thread are seen. At this stage the colonies are indistinguishable from those of the potato bacillus. The edge is fringed with a corona of fine wavy spear points. Liquefaction is rapid, and at the end of twenty hours many of the colonies are 8 mm. in diameter. Each colony soon becomes covered with a thick pellicle, which is a cloudy white, and has irregular ragged edges.

Agar slant.—A scanty granular looking white band forms along the line of inoculation. At the end of four days it has spread to a width of 6 mm., and become white and smooth in the middle, with irregular, granular edges, looking frosted, as it were. After some time it becomes dirty white, and the agar is colored a faint brown.

Gelatin stab.—A small saucer of liquefaction has formed in twenty-four hours, and slight whitish growth is seen along the puncture. The liquefaction assumes the funnel form. The

liquified gelatin has dense white flocculi floating in it, which finally settle to the bottom, leaving the fluid portion almost perfectly clear. The floor becomes level after a time. In cultures kept in an atmosphere of hydrogen, long hair-like outgrowths from the puncture are formed. Smaller ones are sometimes seen in the ordinary cultures also.

Potato.—A dry white layer forms over much of the surface by the third day, which soon becomes a dirty white, smooth, and shining. After two weeks a faint pink color is noticed. The growth does not become folded nor wrinkled.

Bouillon.—Becomes diffusely cloudy by the third day, and a thin pellicle forms on the surface by the fifth day. This becomes thicker, acquires a metallic sheen, and is thrown into large folds.

Rosolic acid.—Becomes lighter about the fifth day. A thin pellicle forms on the surface, and the color is entirely discharged after ten days. The reaction is alkaline.

Litmus milk.—Color becomes lighter and the casein is coagulated in fine flocculi by the fourth day. The color is discharged at the end of a week, and there is a dense shiny deposit at the bottom, apparently casein, that has been changed in some way. Reaction alkaline.

Sugar gelatin, deep stab.—Growth quite deep, with liquefaction as in plain gelatin. No gas formed.

Indol.—Reaction negative.

Relation to temperature.—Grows more rapidly at 35° to 36° C. than at room temperature.

Note.—This bacillus seems to belong to the potato group, and also resembles the *subtilis* in many respects. It is to be distinguished by the formation of its colonies, especially the deep ones, by its lack of motility and the tardy spore formation. From the potato bacillus especially, it may be distinguished by the good growth in hydrogen and its effect on rosolic acid.

Bacillus Viridescens Non-liquefaciens.

Found in made soil, a number of years old, at the depth of 54 inches.

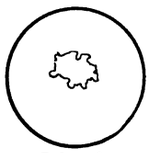
Character.—Good growth in an atmosphere of hydrogen.

Morphology.—Short, straight rods with rounded ends, from two to three times as long as broad. Many of the rods are so short as to be oval. Occurs singly mostly, but also in twos and threes. It stains more deeply at the ends.

Spore formation not observed.

Motility.—Actively motile.

Colonies on gelatin plates.—Colonies appear in thirty-six to forty hours as minute dots, having a faintly greenish tint. At the end of forty-eight hours, the deep colonies are about one-tenth of a millimeter in diameter, while those on the surface are 1 mm. in diameter. × 80. The deep are finely granular, circular in shape, and with even edges. Near the margins fine striæ may be indistinctly seen. The surface colonies have a circular shape, with clean-cut margins, and a faintly green color. × 80. A dense yellowish disk with even margins, in which not much structure can be made out, except near the edges, where it is finely striated. A nucleus, usually eccentric, can generally be made out, and the colony is more dense just around it. Later, the nucleus becomes lost by the increased density, and the edges become finely notched. There is no liquefaction of the gelatin.

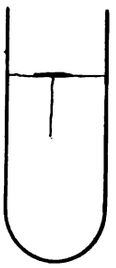


Gel. stab.
Surface growth.

Agar slant.—A very thin greenish white band forms along the line of inoculation by the second day. This spreads near the bottom to the tube walls. It is never thick, and is smooth and glistening, and a very faint greenish white.

Gelatin slant.—A smooth, translucent, greenish white band is formed by the second day with even margins. It becomes more white, is thin and lacks tenacity, so that it flows down to the bottom, collecting in a white mass. It never spreads more than 2 mm., and does not liquefy the gelatin.

Gelatin stab.—The growth down the puncture is scanty, being confined mainly to the surface, where a button 1 mm. in diameter has formed by the second day. This increases until by the end of a week it is 5 mm. in diameter, is white, not very thick, and has irregular leafy edges. There is no liquefaction at the end of four weeks.



Gel. stab.

Potato.—A faintly yellowish, thin layer forms by the second day, and where the potato is moist has spread to the tube wall. It is smooth, moist, and shining. By the fourth day

it has passed all around the plug and become a chocolate brown. Later it becomes dry looking, but is still glistening, and never becomes wrinkled.

Bouillon.—Becomes diffusely cloudy by the third day, and flocculi form on the surface. After two weeks the bouillon becomes a pale green, and a whitish deposit forms at the bottom.

Rosolic acid.—Color becomes darker in four to six days.

Litmus milk.—Becomes more blue by the third day and is a pure blue by the fifth day. Later, a whitish deposit forms at the bottom. There is appearantly no change in the milk itself. Reaction alkaline.

Sugar gelatin, deep stab.—Growth confined mainly to the surface as in plain gelatin. After some time the surface growth become concave, though no softening can be detected. There is no formation of gas.

Indol.—Faint reaction on addition of both sulphuric acid and sodium nitrite.

Relation to temperature.—Grows better at room temperature than at 35° to 36° C.

CLADOTHRIX DICHOTOMA (Cohn).

(Fig. 10, Pl. I.)

Synonym: Brown Cladothrix (Bolton).

Found often in the upper layers of made and virgin soil. Widely distributed. It is also often found in the water of the Schuylkill and other rivers.

Character.—No growth in an atmosphere of hydrogen.

Morphology.—Forms long chains and filaments, with the characteristic false branching which distinguishes the genus. The rods are of very variable length, many of them being club-shaped. Spiral forms are also observed.

Spores have not been demonstrated.

Motility.—Non-motile.

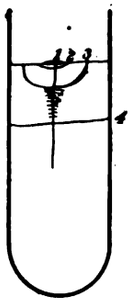
Flagella not demonstrated.

Colonies on gelatin plates.—Colonies appear on the second day as minute white dots. × 80. They are seen to be made up of a tangle of fine lines, the center being so dense that no structure can be positively made out. Toward the edge the lines are wavy and project some distance into the gelatin. At the end of forty-eight hours the deep colonies are one-sixth of a milimeter in diameter, while those on the surface are one-half of a milimeter. Both have acquired a brown color, which extends some distance into the surrounding gelatin. × 80. The deep and surface colonies look much alike, the general structure being the same. The deep colonies grow gradually less dense from the center to the edge, while on the surface there is a tolerably well-defined line of division between the dense central portion and the surrounding corona, marking no doubt the outline of the original growth as it broke through to the surface. Each colony lies in a saucer-shaped depression, and after three to four days a thick brown skin has formed on the liquefied gelatin. At the end of five days the largest colonies are about 3 mm. in diameter, and the brown discoloration extends about 3 mm. into the gelatin, the center being lighter than the surrounding gelatin. × 80. The center is two dense for the structure to be made out, and is brownish in color. The corona is made up of fine wavy lines piercing the gelatin, but they are much shorter in proportion than in the younger colonies.

Agar slant.—At the end of twenty-four hours a whitish line has formed along the inoculation, and the agar has already become a brown tint for 2 or 3 mm. on each side of the growth. The band soon becomes wider and bends across its long axis, sometimes being thrown into wrinkles. It takes firm hold on the agar, and has a dense gristly feel to the needle. It is hard to take up any portion of the growth or to spread it on a cover glass. It has to be broken up by long rubbing and even then many dense masses are left. The agar becomes a beautiful clear brown throughout.

Gelatin stab.—Very slight growth occurs down the line of puncture, and a button forms on the surface, which has begun to sink by the end of twenty-four hours. The gelatin becomes a clear brown. At the end of a week the saucer of liquefaction is about 6 mm. deep, and from the

puncture fine spinous outgrowths may be seen piercing the gelatin. At the end of three weeks, the liquefaction has reached a depth of 1 cm., and apparently the action has ceased. The floor is level, and covered with a whitish flocculent deposit, while the liquefied gelatin is a clear dark brown, the color extending into the solid gelatin for some distance.


Gel. stab.

Potato.—At the end of the third day an elevated, rough, wrinkled band has formed along the line of inoculation. It is of a grayish color, and the potato has turned a deep brown for 2 mm. on both sides of the growth. At the end of four days the growth is 1 mm. high, and the wrinkles have increased. It looks now exactly like a mass of small intestines, closely packed together. Where it has become dry it has turned white on the surface. The potato is colored a dark brown through and through, and the growth can be found in any part of it.

Bouillon.—Growth occurs almost entirely at the bottom in the form of dirty whitish flocculi, while the liquid becomes the color of brandy after some days.

Rosolic acid.—No growth observed.

Litmus milk.—Becomes more blue after two or three days and then grows gradually lighter, and the casein appears to be digested. At the end of three weeks it has become a beautiful violet hue by transmitted light, and in six weeks is a cherry red, a dark brown ring having formed around the tube at the surface.

Sugar gelatin, deep stab.—Growth confined mainly to the surface. Slow liquefaction is caused, and the gelatin becomes brown. No gas is produced.

Indol.—On the addition of both sulphuric acid and sodium nitrite, a reddish color is produced, probably indol.

Relation to temperature.—Growth is more rapid at 35° to 36° C. than at room temperature.

CLADOTHRIX NON-LIQUEFACIENS.

(Figs. 11, 12, 13, and 14, Pl. II.)

Found at the depth of 9 feet in made soil, which had been paved for a number of years.

Character.—Requires oxygen. Shows very slight growth in an atmosphere of hydrogen.

Morphology.—Forms long chains and filaments, which show false branching. No spiral nor coccus forms observed. The filaments break up into rods of various lengths, which have square ends.

Spore formation not observed, though in old cultures some of the rods show unstained areas.

Motility.—Non-motile.

Flagella not demonstrated.

Colonies on gelatin plates.—Colonies appear in thirty-six to forty hours as minute white dots, those on the surface being somewhat larger and yellowish. × 80. The deep have a structure like the branching of a tree, the branches apparently arising from a common center and running outward in every direction to form a circular growth. On the surface the colonies have a central portion of grayish color, made up of fine densely interwoven lines. Toward the edge the lines can be clearly seen in tangled masses, growing thinner and finally breaking out to form a corona of coarse spear points, which are almost straight, and seem to be as large at the ends as when they leave the colony.

The deep colonies grow almost as fast as those on the surface, but after a week become very different in appearance. They are yellowish in color, circular, and not as large as the surface colonies. × 80. They show an opaque center of reddish-yellow hue, surrounded by a beautiful corona of fine wavy and branched lines, which push into the gelatin for a distance nearly equal to the diameter of the colony. The surface colonies at the end of a week are 1 mm. in diameter, are elevated, and nearly hemispherical with white moldy looking surfaces. × 80. They show a dense opaque yellowish center, growing brown toward the edges, where it breaks up into a corona of short, coarse spear points. These bore into the gelatin at tangents crossing each other, forming a coarse basket work which resembles a swallow's nest. After a few days more a second set of finer wavy lines are seen outside of these, and apparently beneath them. At this time

Fig. 11.

Cladothrix non-liquefaciens.
A. Deep colony 44 hrs. old. x 80.

Fig. 12.

Surface colony 44 hrs. old. x 80.

Fig. 13.

Fig. 14.

A. B.

Cladothrix non-liquefaciens.
A. Deep colony 8 days old. x 80.
B. Surface colony 8 days old. x 80.

Fig. 15.

Fig. 16.

A.

Cladothrix Profundus.
A. Deep colony 36 hrs. old. x 80.
B. Surface colony 36 hrs. old. x 80.

B.

Fig. 17.

Bac. Fluorescens Undulatus.
Deep colony 36 hrs. old. x 80.

Fig. 18.

Surface colony 36 hrs. old. x 80.

Fig. 19.

Bac. Striatus Viridis.
3rd. day. x 80.

Fig. 20.

Bac. Striatus Viridis. 4th. day. x 80.

Fig. 21.

Bac. Viscosus.
Deep colony 36 hrs. old. x 80.

Fig. 22.

Bac. Antenniformis.
Deep colonies 14 hrs. old. x 80.

Fig. 23.

Bac. Antenniformis.
Colony which has just reached the surface.
36 hrs. old. x 80.

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the corona of the deep colonies is almost twice the diameter of the central portion. There is no liquefaction of the gelatin.

Agar slant.—A thin whitish layer forms in the course of thirty-six hours. It has smooth edges and is marked by yellowish points. After a few days the yellow growth predominates, and an elevated band 1 mm. high is formed, about salmon color. On the surface it is almost covered with a white mouldy looking growth, which is dry and thin and easily scooped off. Under the microscope it is seen to be made up of short rods and filaments, many of which show false branching. A fine whitish mossy-looking growth is seen pushing into the agar from the under surface of the layer, which appears to be made up of numberless filaments closely packed. In some cultures the salmon-colored layer is absent, the growth being limited to beneath the surface of the agar, with a white, dry, very thin layer on the surface. This is easily scraped off, leaving the surface of the agar perfectly smooth. The conditions which cause this difference of growth have not been clearly made out.

Gelatin stab.—Growth occurs along the puncture and is seen in twenty-four hours, and a white dry looking button forms on the surface, which attains a diameter of 5 mm. by the end of a week. At the same time fine feathery looking offshoots are seen passing out from the puncture. No liquefaction takes place. A very slight brownish discoloration of the gelatin is caused near the surface.

Gel. stab.

Potato.—A yellowish growth, marked with whitish points, is formed along the line of inoculation. It is rough and dry looking, and soon becomes thrown into numberless fine folds and wrinkles. It acquires a pinkish tinge. Is never very abundant.

Bouillon.—A thin film with white powdery looking matter on it is formed in thirty-six hours. The liquid remains almost perfectly clear, while whitish flocculi collect at the bottom.

Rosolic acid.—Growth very scanty. No change in color.

Litmus milk.—The milk becomes more blue by the third day. A thick yellowish ring forms around the tube at the surface. There is no change apparently in the milk itself. Reaction alkaline.

Sugar gelatin, deep stab.—Growth down stab and on surface, as in plain gelatin.

Indol.—Reaction negative.

Relation to temperature.—More rapid growth at 35° to 36° C. than at room temperature.

Cladothrix Profundus.

(Figs. 15 and 16, Pl. II.)

Found at the depth of 9 feet in made soil, which had been paved over for a number of years.

Character.—Requires oxygen. No growth in an atmosphere of hydrogen.

Morphology.—Forms long chains and filaments, which show the characteristic false branching.

Spore formation not observed.

Motility.—Non-motile.

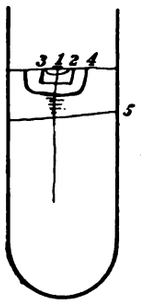
Flagella not demonstrated.

Colonies on gelatin plates.—Colonies become visible in from twenty-four to twenty-eight hours as minute circular white dots. At the end of thirty-six hours the surface colonies are one fourth of a millimeter in diameter; the deep still punctiform. ×80. The deep are made up of very fine wavy lines running radially from the center into the gelatin. The surface colonies are of the same general structure, but the center is too dense to be made out clearly. It is brownish in color, surrounded by a beautiful corona of long, wavy, fine lines, quite regularly disposed. At the end of forty-eight hours the gelatin has taken on a clear brownish hue, and each colony is in a small saucer of liquefaction. As the surface colonies grow older, the center becomes a mottled brown, but no structure can be made out. Surrounding this is a darker zone, made up of densely matted fibers, from which the corona is given off. It is much narrower in proportion than in the younger colonies. A whitish skin, folded in a radial direction, forms after some days on the liquefied gelatin. At the end of five or six days the entire plate becomes liquefied, if it contains

many colonies, and the colonies float in it as small circular islands. Where the plate contains only a few colonies, each may attain a diameter of 3 mm. The liquefied gelatin is brown in color, and the color penetrates the unliquefied portion for about 2 mm.

Agar slant.—A rough, grayish, glistening band, with irregular beaded edges, is formed in the course of thirty-six hours. As it increases it bends in a transverse direction, and along the line of inoculation becomes finely wrinkled, the edges being thinner and undulating. The surface is a bluish gray and has a metallic luster. The growth takes firm hold on the agar, and forms a tenacious leathery membrane. The agar becomes a clear brown.

Gelatin stab.—Growth occurs only in the upper part of the puncture, and on the surface a small button forms. By the third day this has begun to sink and from the puncture fine outgrowths are seen. The growth on the surface becomes white, while the gelatin grows brown for some distance down. The depression in the gelatin has vertical walls, as if punched out. The liquefaction is slow and apparently ceases when a depth of about 2 cm. has been reached. The floor becomes level and has some grayish deposit on it. The liquefied gelatin is a clear brown and the color extends some distance into the solid portion below.



Gel. stab.

Potato.—No growth is seen until the third day, when a yellowish discoloration is noticed. By the next day a finely wrinkled, almost colorless band has formed, and the potato shows a brown discoloration for some distance from each border. It spreads over much of the surface, acquires a grayish hue, and resembles a coil of small intestines very closely. It dries after a time into a thin brownish layer, showing many fine wrinkles, having lost the resemblance to intestines. The potato is never much discolored.

Bouillon.—Growth is confined to the bottom, and around the edges at the surface, while a few flakes of grayish color form on the surface. The bouillon becomes a dark sherry-wine color.

Rosolic acid.—No growth apparently.

Litmus milk.—No change is noticed for some days, when it becomes violet colo . This is slowly changed, and at the end of six weeks has become a plum color. The casein seems to be dissolved, and a whitish deposit forms on the bottom of the tube. Reaction strongly alkaline.

Sugar gelatin, deep stab.—Growth is confined to the upper third of puncture and to the surface. Liquefaction is much more rapid than in plain gelatin, and there is not nearly so much discoloration. A wrinkled yellowish membrane forms on the walls of the depression caused by the liquefaction. There is no gas produced.

Indol.—Reaction negative.

Relation to temperature.—Grows more rapidly at 35° to 36° C. than at room temperature.

Cladothrix Intestinalis.

Found in virgin soil at a depth of 6 feet.

Character.—No growth in an atmosphere of hydrogen.

Morphology.—Forms long filaments, which break up into rods of different lengths. The false branching is seen, characteristic of the genus. The filaments have "buds" on them here and there, which are almost spherical.

Spore formation not observed.

Motility.—Non-motile.

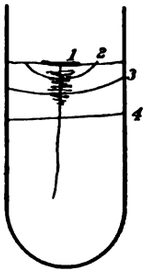
Flagella not demonstrated.

Colonies on gelatin plates.—Colonies appear in from thirty-six to forty hours as minute white dots, those on the surface being somewhat larger. ×80. Deep are made up of branching filaments, running from a common center in every direction. On the surface the colonies show a central portion made up of densely packed filaments, the growth being too dense to show the structure clearly. Toward the edge the filaments are well seen, lying in tangled masses, which break up to form a corona of coarse and somewhat wavy spear points. The color is gray. As the colonies grow older, they become more dense in the center, while the corona of the deep colonies becomes much longer in proportion to the diameter. The filaments are seen to branch distinctly. The surface colonies become white and somewhat mouldy looking, and the gelatin is

slowly liquefied and takes on a brown color. ×80. They do not show much change. The center becomes more dense, and the corona is not so deep in proportion to the diameter of the colony.

Agar slant.—A whitish or grayish beaded growth is seen on the second day. After four days it has become 2 mm. wide, is rough and wrinkled, and the color of dead skin after maceration in water. The wrinkles increase, and after some days the growth resembles a coil of intestines. It does not take firm hold on the agar, and is rather friable. It can not, however, be spread, holding together in small tough masses. The agar is but little discolored.

Gelatin stab.—Growth occurs along the puncture, and on the surface a white mouldy-looking button forms. After five or six days this folds in a radial direction and the edges become somewhat wavy in outline. From the line of puncture beautifully fine feathery outgrowths are seen at the end of a week. Liquefaction occurs slowly, and after six weeks the floor has become level some 2 cm. below the surface. There is some brownish discoloration of the liquefied gelatin, but not much. The button floats on the surface.



Gel. stab.

Potato.—At the end of twenty-four hours only a slight brownish discoloration of the potato is noticed. By the next day a thin layer about the color of the potato has formed, and is much folded and wrinkled. It increases in thickness and the folds become more closely packed together, so at the end of four or five days it resembles a lot of very small intestines very closely—more so than the culture on agar. The potato is only slightly discolored. It soon becomes dry and turns white, as if covered with a fine mould.

Bouillon.—White flocculi settle to the bottom, and the bouillon becomes the color of dark sherry wine at the end of a week.

Rosolic acid.—No growth.

Litmus milk.—Becomes lighter blue in three or four days, a thick pellicle forms on the surface, and a dirty brown ring around the tube. At the end of a week it has become a beautiful violet hue by transmitted light. The color is slowly discharged, but is still present at the end of eight weeks. The casein seems to be dissolved without previous coagulation. Reaction decidedly alkaline.

Sugar gelatin, deep stab.—A fine feathery growth along the puncture and button on surface, as in plain gelatin, with slow liquefaction. There is no discoloration, and no gas produced.

Indol.—The peptone solution becomes the color of pale sherry wine, which interferes with the observation of the reaction. On the addition of both sulphuric acid and sodium nitrite a reddish color is produced, probably due to indol.

Relation to temperature.—More rapid and abundant growth at 35° to 36° C. than at room temperature.

Cladothrix Fungiformis.

Found at the depth of 5 feet in virgin soil.

Character.—Requires oxygen. No growth in an atmosphere of hydrogen.

Morphology.—Forms quite long chains and filaments, which show false branching, not always easily made out.

Spore formation not observed.

Motility.—Non-motile.

Flagella not demonstrated.

Colonies on gelatin plates.—Colonies become visible in about forty hours as minute white dots. × 80. Deep colonies are made up of branching filaments running out in every direction from a common center. The surface colonies have a central portion, made up of densely packed and interwoven filaments, which is bordered by a corona of fine branching filaments. Both deep and surface colonies are gray. At the end of a week the surface colonies are about 1 mm. in diameter, while the deep are somewhat larger. Those on the surface are white and mouldy looking, and have sunk into the gelatin, the depressions looking punched out. Not much structure can be made out under the microscope. The deep colonies show the same general structure, a yellowish center, which has become too dense to show the network of lines of which it is made up, surrounded by a corona of fine filaments, often interwoven and tangled. The gelatin does not become discolored

Agar slant.—A whitish, elevated growth, which soon becomes almost pure white and mouldy looking, is formed in the course of three or four days, and takes firm hold on the agar. After some time it splits here and there, and shows a dark-brown underlayer, the edges of the fissured crusts curling back a little. It has a strong smell of rotten wood. At the end of three or four weeks the agar turns slightly darker.

Gelatin stab.—By the third day there is good growth down the puncture, fine outgrowths having formed for some distance. A white button forms on the surface, and under this slow liquefaction goes on. The depression has at first a punched-out look, with perpendicular walls. It finally reaches the tube wall in about eight days, leaving a ring of gelatin attached to the tube at the surface. The liquefied gelatin is almost clear, and the floor has whitish masses on it. There is no discoloration of the gelatin observed.

Potato.—At the end of three days a scanty almost colorless growth has formed along the line of inoculation, and the potato is distinctly whiter for a distance of 1.5 mm. along each border. The growth becomes a faint yellow, but is always very scanty and thin.

Bouillon.—The liquid remains clear, but becomes slightly darker. A mass of whitish ball-like flocculi collects at the bottom, and colonies about 1 mm. in diameter form around the tube at the surface.

Rosolic acid.—No growth.

Litmus milk.—Becomes more blue by the third day, and a mass of growth floats on the surface. It slowly acquired a beautiful violet tint by transmitted light, and at the end of four weeks has changed to a cherry color. The casein seems to be dissolved without previous coagulation. The reaction is strongly alkaline.

Sugar gelatin, deep stab.—Growth as in plain gelatin. No gas production.

Indol.—Slight color after standing several hours with both sulphuric acid and sodium nitrite.

Relation to temperature.—Grows more rapidly at 35° to 36° C. than at room temperature.

Bacillus Fluorescens Undulatus.

(Figs. 17 and 18, Pl. II.)

Found at a depth of 14 inches in made soil a number of years old.

Character.—Requires oxygen for its full development, though there is some growth in an atmosphere of hydrogen.

Morphology.—Slender, straight rods with rounded ends, from seven to eleven times as long as broad. Forms long chains.

Spores are small and oval in form.

Motility.—Actively motile.

Flagella are situated at the poles.

Colonies on gelatin plates.—The colonies appear in about thirty hours as greenish dots. × 80. Deep colonies are dense-looking brown or gray disks, darker in the center than at the edges, where masses of fine wavy lines can be made out. The surface colonies look much like drops of moisture at the end of thirty-six hours, and have a greenish hue. × 80. They are gray disks, with a well defined nucleus, eccentrically placed and somewhat granular looking. Around the nucleus, and apparently coming out of it, is a zone composed of the most exquisitely fine hair lines, wavy and packed closely together. At the end of three days the surface colonies are 1 mm. in diameter and do not increase in size, but become more dense and elevated. The surface is convex, white in color, with a greenish, iridescent hue. The deep never become larger than one-fourth of a millimeter. × 80. The deep, after a week, are much the same as described above, but more dense and granular. The edges are well defined and even. Those on the surface have become so dense that the structure can not be made out except near the margins, where fine striæ are seen. The nucleus remains visible. There is no liquefaction of the gelatin, and it acquires a faint green color around the colonies.

Agar slant.—In twenty-four hours a thin, translucent, greenish band 2 mm. long has formed along the line of inoculation. It never becomes thick, and after four or five days has spread to the tube wall in a very thin layer for the lower third. It has dentate margins. The color is imparted to the agar—a faint clear green.

Gelatin slant.—An elevated band with even margins forms in thirty-six hours. It becomes elevated, white, and has a greenish, iridescent surface. There is no liquefaction.

Gelatin stab.—Very slight growth along the puncture. A button forms on the surface, which attains a diameter of 4 mm. It is white, shining, and has irregular, leafy margins. There is no liquefaction, and the gelatin may become faintly tinged with green near the surface.

Potato.—A yellowish, moist layer spreads over much of the surface by the third day, and all around the plug at the bottom. It soon becomes a dirty, yellowish brown, is always thin, and has a glossy moist surface.

Bouillon.—Diffuse, but slight cloudiness is seen by the second day, which increases to some extent, but the growth settles largely to the bottom. After some days the bouillon acquires a faint green tint, and at the end of two weeks is a pale clear green.

Rosolic acid.—Very slight growth. After three weeks the color has become darker.

Litmus milk.—Becomes more blue after three or four days. At the end of three weeks it is a deep pure blue. There is no coagulation of the casein or any apparent change in the milk itself.

Sugar gelatin, deep stab.—A button forms on the surface; no growth along the puncture. There is no gas produced.

Indol.—Reaction negative.

Relation to temperature.—Grows better at room temperature than at 35° to 36° C.

Micrococcus Putatus.

Found in made soil at a depth of 38 inches.

Character.—Strict aërobe.

Morphology.—Large coccus forming irregular groups.

Spore formation not observed.

Motility.—Non-motile.

Flagella not demonstrated.

Colonies on gelatin plates.—Colonies appear in from forty to forty-eight hours as minute yellowish dots, which easily escape observation, being translucent. On the surface they are only one-tenth of a millimeter in diamater. × 80. Deep are round, finely granular, and have even edges. Those on the surface are round, with regular edges, and have a yellowish cast. They are finely granular, and have an eccentric nucleus, of slightly darker hue. After five days, the deep colonies are still punctiform, while those on the surface are one-half of a millimeter in diameter, and have a greenish yellow color. × 80. Deep have become more dense, but otherwise are unchanged. The surface are yellowish disks, with even distinct edges. No structure can be made out except near the edges, where fine wavy lines radially disposed are seen. The nucleus still remains visible. At the end of a week the colonies are distinctly yellow, but never become larger than 1 mm. Slow liquefaction occurs after two weeks.

Agar slant.—A beaded line forms along the needle stroke in twenty-four hours, of a faint yellowish color. It increases rather slowly, and becomes a canary yellow after ten or twelve days. The surface becomes smooth and glistening, and the edges of the band are notched.

Gelatin slant.—A band 1 mm. wide forms along the line of inoculation, of a yellowish hue. At the end of four days the canary yellow color is well developed. After two or three weeks the gelatin is slowly liquefied, and the growth slips slowly down the shallow groove which is formed.

Gelatin stab.—Growth is confined to the upper part of the puncture, and by the fourth day a button has formed on the surface, which reaches a diameter of 4 mm. at the end of ten days. It is quite thin, yellow in color, and has even margins. Liquefaction begins about the twelfth day, and the surface growth becomes concave, then slowly sinks.

Potato.—A yellow layer is formed by the third day, spreading at the bottom. After ten days a dense, abundant growth has formed, reaching the walls of the tube for the lower half. It is smooth, moist, and shining, and lemon yellow in color.

Bouillon.—The liquid becomes slightly cloudy by the third day, but the growth is confined almost entirely to the bottom, where it forms granular-looking yellowish masses, not abundant.

Rosolic acid.—Growth is scanty. Color is somewhat darker after a week.

Litmus milk.—Becomes more blue by the third day. After a week is a pure blue. There is no coagulation or any change apparent in the milk itself. A yellowish deposit forms at the bottom of the tube.

Sugar gelatin, deep stab.—Very scanty growth, and confined to the surface mainly. No gas is produced.

Indol.—Reaction negative.

Relation to temperature.—Almost no growth at 35° to 36° C.

Bacillus Striatus Viridis.

(Figs. 19 and 20, Pl. II.)

Found at a depth of 18 inches in made soil a number of years old.

Character.—No growth at all in an atmosphere of hydrogen.

Morphology.—Straight, rather slender rods, of variable length, with rounded ends. Each rod is marked by transverse bands which do not take the stain, giving it a striated appearance, not unlike the bacillus *Diphtheriæ*. Occurs singly for the most part, though pairs are seen also.

Spores.—Formation of spores is doubtful.

Motility.—Actively motile. Occasionally one or more rods will dart across the field with a rapidity which makes it hard to follow them.

Flagella are situated at the poles.

Colonies on gelatin plates.—Colonies appear at the end of thirty hours as minute bluish-white, translucent dots, about one-sixth of a millimeter in diameter. Deep colonies are punctiform. × 80. Deep are yellowish, finely granular, with circular even edges. Those on the surface have a yellow hue, and three distinct zones can be made out, the color growing paler from the center outward. Growth is slow, and at the end of five days the surface colonies are only 1 mm. in diameter, the deep remaining whitish dots. × 80. Deep appear as dense brown disks with even edges. The surface colonies still show the zones. Just outside of the zone surrounding the nucleus have appeared brownish masses, with clearly defined edges, heaped irregularly on each other, resembling amorphous crystals. The nucleus is usually eccentric. In some colonies these amorphous granules are almost all grouped at one side of the colony, but usually they form a more or less regular circle. (See drawing.) After a few days the colonies become grayish and lose their even margin, a fringe of fine wavy lines having formed. The granules have become more numerous and are more regularly disposed.

Agar slant.—By the second day a smooth, shining band, with irregular margins has formed, which spreads near the bottom in a very thin almost invisible layer, which has leafy margins. The agar acquires a faint green tint by the third day, which later becomes a yellowish green. The growth soon covers much of the surface of the agar, but is never very thick, and becomes somewhat shiny.

Gelatin stab.—Growth is quite slow, and confined mainly to the surface, where a whitish button, some 2 mm. in diameter, is formed by the end of a week, and later becomes double that size. It has even, elevated margins and is white. There is no liquefaction of the gelatin.

Potato.—By the second day there is an abundant growth, spreading widely over the plug where it is moist. It becomes a chocolate brown after two weeks, and is moist and shining.

Bouillon.—Diffusely cloudy at the end of twenty-four hours. Becomes a faint green at the end of a week and the growth settles to the bottom as a white mass.

Rosolic acid.—No change in color is produced.

Litmus milk.—No change is seen until about the tenth day, when it becomes more blue, without any coagulation. After five weeks, the color has been entirely discharged.

Sugar gelatin, deep stab.—Growth very scanty and mostly on the surface, where a button forms. There is no liquefaction and no production of gas.

Indol.—Reaction negative.

Relation to temperature.—Grows well at both room temperature and at 35° to 36° C.

BACILLUS VISCOSUS (Frankland).

(Fig. 21, Pl. II.)

Found in meadow soil at the depth of 30 inches.

Character.—Very slight growth in an atmosphere of hydrogen.

Morphology.—Small, straight rods with rounded ends, from three to five times as long as broad. Occurs singly and in pairs.

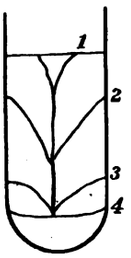
Motility.—Actively motile.

Flagella situated at the poles.

Spores not observed.

Colonies on gelatin plates.—Colonies appear in twelve to fourteen hours as minute whitish dots, barely visible to the naked eye. × 80. They are finely granular, grayish disks, with even margins. At the end of eighteen hours the surface colonies are one-half of a millimeter in diameter, circular and whitish, each lying in a small saucer of liquefaction. × 80. They are made up of masses of fine wavy lines, giving a fringed appearance to the margins which are uneven, though generally circular in shape. Liquefaction is rapid, and at the end of thirty-six hours the surface colonies are 6 to 8 mm. in diameter, the liquefied gelatin containing grayish flocculi. The edges are clear cut and circular. × 80. The flocculi are made up of fine threads in tangled masses. At the edge these lines assume an outward direction, forming a corona of fine spear points, the colonies at this stage resembling those of the subtilis very closely. The deep colonies are one-sixth of a millimeter in diameter, and whitish in color. × 80. The central portion is yellowish brown and granular, and is surrounded by a corona of fine wavy lines, whose length is about equal to the diameter of the central part of the colony. Liquefaction is rapid on the surface, and further observation is impracticable.

Agar slant.—A thin, smooth, glistening, greenish line is formed along the needle mark at the end of twenty-four hours. This soon spreads over a large part of the surface, and the agar acquires a green tint.



Gel. stab.

Gelatin stab.—Growth occurs down the puncture, and a funnel of liquefaction is formed by the second day, in which whitish flocculi are floating. By the third day the gelatin is liquefied to a depth of 1 cm., measured along the tube wall, and the floor is becoming less funnel shaped. A tenacious layer forms on the surface about the fifth day, and the whitish deposit at the bottom is also tenacious. The liquefaction proceeds nearly to the bottom, and the floor becomes level, after which the action seems to cease.

Potato.—A smooth, moist layer of yellowish hue forms in twenty-four hours. This soon spreads over the entire surface of the plug in a thin layer, and becomes a chocolate brown about the tenth day. It is smooth, moist, and shining.

Bouillon.—Diffuse cloudiness, not very intense, is caused by the third day. The bouillon becomes green at the surface about the same time, the color extending downward, so that about the tenth day it is green all through. A whitish deposit forms at the bottom.

Rosolic acid.—A slight film forms on the surface, but no change occurs in the color.

Litmus milk.—Becomes reddish about the fifth day, but no coagulation can be detected. Later the milk becomes watery, apparently from digestion of the casein, and a white deposit collects at the bottom.

Sugar gelatin, deep stab.—Growth occurs deep along the puncture, with rapid liquefaction, and a layer forms on the surface. No gas is produced.

Indol.—Reaction negative. An odor like skatol is produced by boiling with sulphuric acid and sodium nitrite.

Relation to temperature.—More rapid at 35° to 36° C.

Note.—This bacillus was first described by Grace and Percy Frankland in the Zeitschrift für Hygiene, Vol. VI, 1889.

BACILLUS VIRIDESCENS LIQUEFACIENS.

Found in meadow soil at a depth of 30 inches.

Character.—Requires oxygen for its full development, though some growth occurs in an atmosphere of hydrogen.

Morphology.—Small, straight rods, with rounded ends, from three to five times as long as broad. Occurs singly.

Spores are not formed until about the tenth day. They are small ovals and formed in the center of the rods.

Motility.—Active movements.

Flagella situated at the poles.

Colonies on gelatin plates.—Colonies seen at the end of twelve hours as minute whitish dots, with a bluish cast. × 80. They are translucent disks, looking like drops of moisture, with even edges. At twenty hours they are more white and the largest one-half of a millimeter in diameter, and each one is already beginning to form a saucer of liquefaction. × 80. Both deep and surface colonies appear as grayish, granular disks, with well-defined but irregular margins. On the surface the margins are soon lost, and the colony appears as a mass of fine wavy lines, twisted and curled. The central portion is made up of cloudy masses of a grayish hue. Some of the deep colonies have a lobed appearance, the center appearing as if higher than the edges, which have a fissured look. Liquefaction proceeds very rapidly, and the colonies lose any characteristic form very soon.

Agar slant.—A smooth, elevated, greenish-white band, spreading widely at the bottom, is formed by the second day. At 35° to 36° C. the agar is largely covered in twenty hours, and has already acquired a green tint, which only comes at room temperature about the third day. The growth spreads from the edges in a very thin layer with uneven margins, and becomes a beautiful yellowish green. It is white in the water at the bottom.

Gelatin stab.—Good growth is seen down the puncture in twenty-four hours, and at the surface a funnel of liquefaction has formed, whitish flocculi sinking to the bottom. By the third day the liquefaction has reached a depth of 1 cm., measured at the tube wall, the floor becoming less funnel shaped. By the fourth day more than two-thirds of the gelatin has been liquefied. The floor becomes level after a few days more and covered with white flocculi. The process seems to stop, or else proceeds very slowly. The liquefied gelatin does not become green.

Potato.—An abundant yellowish growth, spreading to the walls of the tube where moist, is seen on the second day. It is moist and shining, not very thick. After five or six days it becomes a brownish color.

Bouillon.—Diffuse cloudiness on second day. Green tint is caused near the surface by the end of the third day, and soon the whole of the liquid becomes green. On the bottom of the tube an abundant white deposit collects.

Rosolic acid.—A thin film forms on the surface, but no change in color is caused.

Litmus milk.—Reddish tinge appears in forty-eight hours, but no coagulation of the casein is observed. A scum forms on the surface and a whitish deposit at the bottom. After some days the milk becomes watery, apparently from digestion of the casein.

Sugar gelatin, deep stab.—Grows to bottom of puncture and causes rapid liquefaction. No gas produced.

Indol.—Reaction negative. On boiling after the addition of sulphuric acid and sodium nitrite an odor like skatol is produced.

Relation to temperature.—More rapid at 35° to 36° C.

Note.—This bacillus resembles the *Fluorescens liquefaciens* very closely, and may be identical with it. The chief difference noted, as far as the published descriptions go, is in the production of spores. No culture of the bacillus *F. liquefaciens* was at hand with which it could be compared.

BACILLUS RAMOSUS.

Synonyms: Bacillus Mycoides (Flügge). Root form bacillus (Fränkel); Wurtzel bacillus.

Character.—Aërobe. Found constantly in both made and virgin soils, both near the surface and at a considerable depth.

Morphology.—Thick, straight rods with rounded ends, from two to three times as long as broad. Forms chains of considerable length.

Spores are large ovals, formed about the center of the rods.

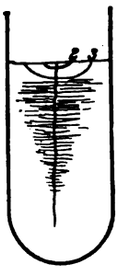
Motility.—Movements are sluggish.

Flagella not demonstrated.

Colonies on gelatin plates.—Colonies are well advanced in sixteen hours. They are indistinct cloudy masses, with ill-defined margins, resembling the mycelium of a fungus somewhat. × 80. The center is composed of fine tangled lines, which send out long filaments in every direction, forming a circular mass. They are much tangled and twisted. At the end of twenty-four hours the colonies are more distinct, and 4 mm. in diameter. The gelatin has become softer, and has an iridescent gleam when viewed slantingly. By the third day the colonies are more than 1 cm. in diameter if there be but few on the plate. The gelatin does not pour as when liquefied by the *Subtilis*, for example, being held in the interstices of the dense network of fibers. × 80. But little more is seen than on the first day. The fibers have multiplied immensely and are closely matted together, giving a grayish appearance.

Agar slant.—A moist looking, grayish growth forms in twenty-four hours along the line of inoculation, which has a somewhat felted look. From the edges fine fibers run out and soon reach the tube wall. The whole mass thickens, forming a grayish layer, which often wrinkles in the middle. The folds are best seen by looking at the under surface.

Gelatin stab.—A small saucer of liquefaction is formed on the surface by the second day, and very soon fine filaments are seen boring into the gelatin in every direction, giving the appearance of "an inverted fir tree." A moist, shining skin is formed on the surface, which becomes folded and slowly sinks, a second one forming. The liquefaction becomes complete after some days, and the cloudy masses which floated in the liquid sink to the bottom.


Gel. stab.

Potato.—A white granular-looking layer, which covers much of the plug is formed in the course of four days. This becomes much folded and wrinkled if the potato has been freshly prepared.

Bouillon.—Good growth in the form of veil-like masses, which resemble closely bits of Japanese tissue paper sunk in the liquid. The bouillon does not become cloudy.

Rosolic acid.—Color is deepened in thirty hours, and becomes decidedly deeper in ten days.

Litmus milk.—Casein is precipitated as a jelly-like mass in twenty-four hours, and the color is slightly darker. The blue color is lost from above downward, the clot becoming the color of milk. It is digested from above downward also, the process being complete in about two weeks. It is then the color of whey, and a blue ring is seen around the tube at the surface. The reaction is decidedly alkaline.

Sugar gelatin, deep stab.—Growth quite deep down the puncture, with formation of fine outgrowths, as in plain gelatin. No gas is produced.

Indol.—Reaction negative.

Relation to temperature.—Grows well at room temperature, and also at 35° to 36° C.

Bacillus Antenniformis.

(Figs. 22 and 23, Pl. II.)

Found at the depth of 5 feet, in virgin soil used as a meadow.

Character.—No growth in an atmosphere of hydrogen.

Morphology.—Large, straight rods, with rounded ends, from eight to ten times as long as broad. Occurs singly for most part.

Motility.—Actively motile.

Flagella not demonstrated.

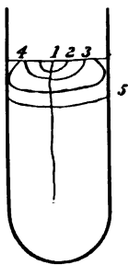
Spore formation not observed.

Colonies on gelatin plates.—Colonies are seen in from twelve to fourteen hours as minute colorless dots. × 80. They are slightly oval, yellowish, and granular. From various portions of the periphery, mainly near the poles of the oval, short fine projections are seen, resembling

the antennæ of insects. At the end of thirty-six hours these can not be seen and the resemblance is lost. At this time the surface colonies are one-fourth of a millimeter in diameter, and are grayish. × 80. They have an orange-brown center, which sometimes shows a granular nucleus. Around it is a coarsely granular zone of the same color, which passes into a fringe of wavy lines, beyond which is a border of colorless markings apparently made up of parallel lines closely packed and folding in and out. It is deeply dentate, and so faint as to be easily overlooked. As the colonies grow older, the granular center encroaches on the zone of the wavy lines and they in turn on the border, which becomes more regular in outline and narrower in proportion to the size of the colony. Each colony causes a saucer of liquefaction on which a pellicle forms, which soon becomes folded. Liquefaction is not very rapid. At the end of a week the colonies are about 6 millimeters in diameter. Under 80 not much can be made out except near the edges, where they have a reticular appearance. They are circular and have well-defined edges.

Agar slant.—On the second day a very thin, barely visible band with irregular margins is seen, which near the bottom has spread considerably in leafy shapes. It soon spreads over the entire surface of the agar, except near the top. It is very thin, smooth, translucent, and grayish.

Gelatin stab.—Slight development down the stab near the surface. On the third day a saucer of liquefaction 2 mm. in diameter has formed, which soon has a pellicle on its surface. The liquefaction reaches the wall of the tube under the surface of the gelatin, leaving a ring attached to the tube. The floor becomes level by the tenth day, 1 cm. from surface, and has a light deposit of whitish flocculi on it. The liquid is cloudy. Further liquefaction is very slow.



Gel. stab.

Potato.—"Invisible" on the second day. A moisture only is seen along the line of inoculation, spreading near the bottom to the tube wall. By the third day much of the surface is covered and the layer is thrown into fine folds, which resemble herpetic vesicles at some points. It becomes putty-colored and drier looking, and the folds more numerous. It is always very thin.

Bouillon.—Growth is never abundant, and soon settles to the bottom, a few flakes floating on the surface.

Rosolic acid.—Very slight growth and barely perceptible deepening of the color.

Litmus milk.—No change until fifth day, when the color becomes lighter without redness. It is discharged in ten to twelve days, and the milk becomes watery. No coagulation of the casein observed. Reaction faintly acid.

Sugar gelatin, deep stab.—Growth is scant deep down, and not seen until three or four days. Liquefaction takes place at the surface. No gas produced.

Indol.—Reaction negative.

Relation to temperature.—Grows well at room temperature, but somewhat faster at 35° to 36° C.

Bacillus Trommelschlägel.

Found at the depth of 4 feet in made soil, which had been paved over for several years.

Character.—Grows fairly well in an atmosphere of hydrogen.

Morphology.—Slender, straight rods with rounded ends, from five to seven times as long as broad. Occurs singly and also forms short chains. In the separate rods, as in the chains, are seen deeply stained dots, from one to three in each rod. In the chains the line of division between the separate elements is often impossible to make out.

Spores are formed in eighteen to twenty hours at 35° to 36° C. They are round or slightly oval and formed at the ends, giving the most perfect form of the drumstick. The spore is not exactly at the end, as a small part of the rod, which is usually pointed, projects beyond it. The best specimens are obtained from agar cultures, at room temperature, on the third day.

Motility.—Movements are active and progressive. Spore-bearing rods are non-motile.

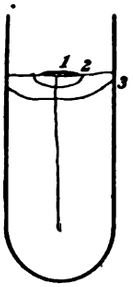
Flagella are attached at the poles.

Colonies on gelatin.—Colonies seen at the end of twenty to twenty-four hours. Deep are whitish and punctiform, and those on the surface but little larger. × 80. Deep are irregularly circular, yellowish, granular disks, with even edges. The surface colonies show a nucleus, usually

placed to one side of the center. Around the nucleus is a yellowish, granular zone, which in turn is surrounded by a grayish zone, also granular, and which has very irregular edges. Both are finely veined. After several days the surface colonies attain a diameter of 1 mm., and become porcelain white and elevated, with even edges. The deep do not get much larger, but become more dense and whiter. × 80. There is no change seen in the deep colonies. Those on the surface become too dense to show much structure. They appear as gray disks with regular margins, and near the margins have a finely granular and veined appearance. There is nothing at all distinctive about the colonies. No liquefaction takes place.

Agar slant.—On the second day a very thin, translucent layer has formed, which extends to the tube wall for the lower half. It is smooth, shining, and has a greenish tint. After some days it is somewhat thicker, but always very thin and translucent. After ten days the agar acquires a faint yellowish-green tint, which becomes brownish after three or four weeks.

Gelatin stab.—Growth occurs along the puncture in twenty-four hours. By the third day there has formed a thin white layer on the surface, which has become 4 mm. in diameter by the end of a week. It is white in color, and has irregular, leafy margins. The growth along the puncture has not increased to any extent. Slow liquefaction is observed about this time, and the surface growth sinks slowly. The gelatin below the liquefied portion becomes cloudy after two weeks. At the end of two weeks the liquefaction is only about 2 mm. deep, and reaches the tube wall.



Gel. stab.

Potato.—At the end of twenty-four hours only a widespread moisture can be seen. By the next day it has become like a thin layer of honey. By the fifth day it is brown and dry, and has a metallic luster. No further change takes place.

Bouillon.—A slight, diffuse cloudiness is caused by the second day. At the end of a week most of the growth has settled to the bottom, leaving the liquid tolerably clear.

Rosolic acid.—Becomes slightly darker after two or three days.

Litmus milk.—Becomes darker by the third day, after which the color is gradually discharged. The reaction is alkaline. At the end of two weeks the milk is a dirty white, while a blue zone is formed on the tube wall at the surface. After four weeks the casein seems to be dissolved, the milk becoming watery and translucent.

Sugar gelatin, deep stab.—Growth occurs quite deep along the puncture, and a button forms on the surface, with slow liquefaction. No gas is produced.

Indol.—Reaction negative.

Relation to temperature.—More rapid and abundant at 35° to 36° C.

Bacillus Geminus Major.

Found at a depth of 4 feet in made soil, which had been paved for several years.

Character.—Strict aërobe. No growth in atmosphere of hydrogen.

Morphology.—Straight, thick rods of irregular length, with rounded ends. Occurs singly, though short chains are occasionally found. The rods show deeply stained points, usually situated near the ends, from one to three in each rod.

Spores not observed.

Motility.—Very slight movement.

Flagella not demonstrated.

Colonies on gelatin plates.—Colonies appear in twenty hours as minute whitish dots. × 100. Deep are brownish, granular disks, with even edges. Surface are grayish and wavy looking, and have a wavy outline. Many show nuclei. At the end of forty-two hours the deep are still punctiform. Those on the surface are 1 mm. in diameter and have a greenish cast. × 100. Deep unchanged, except that they have become more dense. The surface are grayish, and have an eccentric nucleus and a wavy outline. They are marked by darker lines irregularly distributed, and joining each other, giving the colony a marbled appearance. The colonies are not unlike those of the typhoid bacillus, but are more granular and coarser. The gelatin becomes somewhat softened after a time, but no distinct liquefaction can be detected.

Agar slant.—A faint band with leafy margins is formed by the second day. It spreads to the tube at the bottom and becomes somewhat thicker, but is always a very thin, translucent layer without color. The deposit in the water at the bottom is white.

Gelatin stab.—Growth occurs quite deep down, and a whitish layer forms on the surface, which is only about 1 mm. in diameter, and has irregular margins. By the third day from the upper part of the stab delicate offshoots pierce the gelatin, which soon reach nearly to the tube wall and resemble the leaves of asparagus. The growth on the surface extends in a barely perceptible layer until, after two weeks, the surface is largely covered. There seems to be some softening at the surface after eighteen or twenty days, but no liquefaction can be made out.

Gel. stab.

Potato.—A thin layer of moisture, very faintly yellowish, is seen on the second day. It becomes thicker, and about the color of honey on the third or fourth day, and is moist, smooth, and shiny. After a week it becomes a chocolate brown, but does not increase in thickness.

Bouillon.—Becomes diffusely cloudy on the third day. After a week the growth begins to settle to the bottom, and the liquid is left tolerably clear.

Rosolic acid.—Becomes slightly darker after ten days.

Litmus milk.—Becomes more blue after a week. At the end of two weeks it is a sky-blue. There is no coagulation of the casein, but a white precipitate collects at the bottom of the tube. After five weeks the color is somewhat lighter, but a pure blue. Reaction is amphoteric.

Sugar gelatin, deep stab.—Growth quite deep down and on the surface. There is more liquefaction than in plain gelatin. No gas is formed.

Indol.—Negative reaction.

Relation to temperature.—More rapid and abundant at 35° to 36° C.

Note.—This bacillus does not form glutinous masses on any culture medium, but seems to have the power of holding other growths to it. The bacillus *Geminus minor* was found with it, and it was with much difficulty that the two were separated. A portion of the growth was placed in Dunham's solution, and repeatedly shaken at intervals for two or three hours, and from this plates were made. It was only after repeated trials that the two were gotten out in pure culture.

Bacillus Geminus Minor.

Found at a depth of 4 feet in made soil, which had been paved for several years, in close union with the bacillus *Geminus major.*

Character.—Requires oxygen for its development.

Morphology.—Very short straight rods, with rounded ends, from two to four times as long as broad. Occurs singly.

Spore formation not observed.

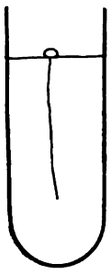
Motility.—Very active movements, like fighting or playing.

Flagella not demonstrated.

Colonies on gelatin plates.—Colonies become visible in twenty-four to thirty hours. At the end of thirty-six hours the deep are whitish punctiform dots, while those on the surface are white also and one-sixth of a millimeter in diameter. × 80. Deep are yellowish in color, coarsely granular, with even margins. The surface colonies are much the same, only showing a nucleus. By the third day the surface colonies have become pearly white, are evenly circular, and have rounded surfaces. They are so dense that little can be seen under the microscope. Near the edges they have a wavy appearance, as if made up of serpentine parallel lines closely packed. After ten days they have become 1.5 mm. in diameter, are more elevated, and are a pearly white. They retain their circular outline and have well-defined edges. They become too dense to show any structure under the microscope. The deep colonies remain unchanged, except to increase in density. There is no liquefaction of the gelatin.

Agar slant.—A thin greenish-white line is formed by the second day. It spreads rather slowly, grows more white, and in eight or ten days has covered much of the surface of the agar in a thin shiny-looking layer, which runs to the bottom.

Gelatin slant.—An elevated porcelain-white band 1 mm. wide, which has a smooth surface and even margins, is formed by the third day. It is raised one-half of a millimeter. After about ten days indistinct striations may be seen on the surface of the gelatin running at right angles to the band of growth, but no growth can be made out positively.



7 days.
Gel. stab.

Gelatin stab.—Good growth occurs quite deep, and on the surface a button forms by the second day. It is 1 mm. in diameter and half as high. After a time the edges, which are thinner, become corrugated, while the center remains smooth and a glistening pearl white. For some distance down puncture delicate, spinous offshoots are seen, not more than one-fourth of a millimeter long. There is no liquefaction.

20 days.
Gel. stab.

Potato.—A faintly yellowish line forms along the needle mark by the second day, which has spread widely, where the potato is moist, in a very thin colorless layer. It increases slowly and becomes by the tenth day a dirty white, smooth, moist, and shining layer.

Bouillon.—Becomes diffusely clouded by the third day, and a thin film forms on the surface. Later, the growth collects at the bottom of the tube.

Rosolic acid.—Becomes cloudy, and by the tenth day is a cherry-red color.

Litmus milk.—More blue by fourth day. After two weeks changes to a slate-blue, and a whitish deposit forms at the bottom. There is no coagulation or any change apparently in the milk itself. The reaction is strongly alkaline.

Sugar gelatin, deep stab.—Growth quite deep down the stab and on the surface. No gas is produced.

Indol.—Good reaction with both acid and sodium nitrite.

Relation to temperature.—Grows more rapidly at 35° to 36° C.

Bacillus Solitarius.

Found at the depth of 4 feet in made soil, which had been paved for several years.

Character.—Strict aërobe. No growth in an atmosphere of hydrogen.

Morphology.—Slender, straight rods, with rounded ends, from three to seven times as long as broad. Occurs singly.

Spore formation not observed.

Motility.—Some rotatory non-progressive movements.

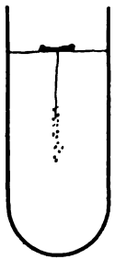
Flagella not demonstrated.

Colonies on gelatin plates.—Colonies become visible in from thirty-six to forty hours, as grayish dots. × 80. Deep are grayish, smooth-looking disks, with finely notched margins, the contents being homogeneous or sometimes finely granular toward the center. Surface colonies present much the same appearance, but are larger, and some irregular bundles of fine lines can be seen. At the end of forty-eight hours the deep colonies show a nucleus, around which are tolerably well-defined rings or zones, growing lighter toward the edge. Fine wavy lines can be seen running in various directions, giving the colony a somewhat marbled appearance. At the end of seventy hours the surface colonies are 1 mm. in diameter, and of a greenish-white color, with even edges. × 80. A granular nucleus is seen, surrounded by an orange-colored zone, made up of fine lines closely packed. Some are so dense that the lines can not be made out. The edges of this zone are not well defined, and are irregular, sending ragged offshoots into the surrounding zone, which is a lighter shade, and in which wavy lines running toward the edge can be seen. At the end of a week the surface colonies are milk white, round, elevated, and have smooth surfaces and even edges. They are too dense to show any structure under the microscope, except near the margins, where wavy lines are seen. Deep have not changed, except to grow more dense. There is no liquefaction of the gelatin.

Agar slant.—By the second day a greenish-white line 1 mm. wide has formed. It grows thicker, spreads almost to the tube wall for the lower third, and becomes much whiter, so that by the fourth day it is porcelain white, has irregular margins, and is smooth, moist, and shining. The growth which collects at the bottom becomes cream colored after some days.

Gelatin slant.—Resembles the growth of the typhoid bacillus very closely. A dry, white line, thin and with iridescent gleam, and later cracks across the surface transverse to the long axis.

Gelatin stab.—Growth is confined to the surface mostly for the first three or four days, forming a button like a drop of milk. By the fourth day there is good development to the bottom of the puncture, being in separate colonies low down, as minute white spheres. The growth on the surface attains a diameter of 4 mm. by the eighth day, and is elevated 1 mm., with a slight central depression. It is porcelain white and has even edges. The gelatin becomes opaque around it, but no growth can be detected with the needle. The button later becomes cream colored. There is no liquefaction.


Gel. stab.

Potato.—At the end of twenty-four hours a line of moisture is seen along the needle mark. By the next day it has spread widely in a thin whitish layer. It increases in thickness, and has a curdled appearance. At the end of two weeks it becomes thick and pasty looking, and the color of putty.

Bouillon.—Becomes diffusely clouded by the third day, and later a dirty-white deposit is seen on the bottom.

Rosolic acid.—Becomes lighter by the third day, and the color is almost entirely discharged at the end of a week. Reaction alkaline.

Litmus milk.—The color becomes a pure blue in three days and then grows darker, until at the end of three weeks it is slate colored. There is no change apparently in the milk itself. A white deposit collects at the bottom.

Sugar gelatin, deep stab.—Growth in separate colonies deep down, with button on the surface. There is no gas produced.

Indol.—Reaction negative.

Relation to temperature.—More rapid at 35° to 36° C.

Note.—This bacillus resembles the *Geminus minor* very closely in many ways. The chief points of diagnosis are the discharging of the color of rosolic acid, while the former changes it to cherry red, and the formation of single colonies in the bottom of gelatin stab cultures.

Bacillus Cinctus.

(Figs. 24, 25, and 26, Pl. III.)

Found at the depth of 4 feet in made soil, which had been paved over for several years.

Character.—Very slight growth in an atmosphere of hydrogen.

Morphology.—Straight rods which vary considerably in size. Involution forms are found in eighteen to twenty hours in agar cultures kept in the incubator. It occurs singly mostly, but chains of several elements are found also, the line of division not being well marked. Each rod has from one to three deeply stained dots in it, placed irregularly, though a majority are at, or near, the poles. These do not seem to have anything to do with spore formation, as they are seen in spore-bearing rods.

Spores are formed in three days at room temperature. They are large ovals, and distend the walls of the cells. Formed near the ends.

Motility.—It is actively and progressively motile.

Flagella not demonstrated.

Colonies on gelatin plates.—Colonies seen in twenty to twenty-four hours as indistinct translucent points, which, under a low power, are yellowish, granular disks, with even margins. Growth is slow, and at the end of seventy hours the deep colonies are still punctiform, while those on the surface are one-half of a millimeter in diameter. The latter are greenish white, with irregular margins. × 80. The surface colonies show a nucleus, eccentrically placed, around which is a zone, yellowish in color, which appears granular at first. A close study shows fine lines so closely laid as to produce this appearance. Outside of this is a zone of gray, which is irregularly veined, and has very irregular margins. There is but little change from day to day. At the end of eight days the surface colonies are only 1 mm. in diameter, greenish white, round, and elevated, with even margins. The deep are about one-sixth of a millimeter in diameter, and grayish. × 80. Deep are a bright yellow with reddish tinge, fading gradually to a grayish color at the margins,

Fig. 24.

Bac. Cinctus.
One form of surface colony 36 hrs. old. x 80.

Fig. 25.

A.

Fig. 26.

B.

Bac. Cinctus.
A. Deep colony 70 hrs. old. x 80.
B. Surface colony 70 hrs. old x 80.

Fig. 27.

Bac. Vacuolatus.
Surface colony 46 hrs. old. x 80.

Fig. 28.

A.

Fig. 29.

B.

Bac. Pinnatus.
A. Deep colony 60 hrs. old. x 80.
B. Surface colony 60 hrs. old. x 80.

Fig. 30.

Bac. Gangliformis.
Deep colony 14 hrs. old. x 80.

Fig. 31.

Bac. Erodens.
Deep colony 36 hrs. old. x 80.

Fig. 32.

Potato Bacillus (mesentericus vulgatus.)
Deep colony. 2nd day. x 80.

Fig. 33.

Potato Bacillus
(mesentericus vulgatus.)
Colony just reached the surface. x 80.

Fig. 34.

Potato Bacillus
(mesentericus vulgatus.)
Large surface colony 3 days old.
x 80.

Fig. 35.

Bac. Ginglymus.
Surface colony 7 days old. x 80.

Fig. 36.

B.

Fig. 37.

A.

Bac. Fissuratus.
A. Deep colony 16 hrs. old. x 80.
B. Deep colony 36 hrs. old. x 80.

Fig. 38.

Bac. Arborescens non-liquefaciens.
Colony 60 hrs. old, showing other forms. x 80.

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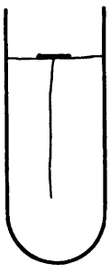
showing several zones or concentric circles. The center is indistinctly veined and granular, the edges smooth and clear cut. The cause of the zones is not apparent, as there is no abrupt change in color or density. The surface colonies still show a nucleus, surrounded by a reddish-yellow zone, which has smooth edges and fades evenly from center to margin. It is finely veined and sometimes mottled. Some colonies have a striking resemblance to the shell of an oyster. When there are very few on the plate, grotesque forms are produced after a time, ferny outgrowths going from the margins. These spread in most irregular shapes, later inclosing islands of gelatin, by the projections meeting each other. They attain a diameter of 4 mm.

Agar slant.—A thin, glistening, pale green layer forms along the line of inoculation which has spread to the tube wall for the lower half by the second day. It increases somewhat, but is always thin and translucent, with smooth surface. The agar becomes a faint yellow.

Gelatin slant.—A white band is formed, 1 mm. wide, with rather uneven edges. After a time it cracks across the long axis, and the gelatin seems somewhat softened at the end of five weeks, but no liquefaction occurs.

Gelatin stab.—Growth occurs along puncture, and a white layer forms on the surface, which has very irregular leafy margins, and attains a diameter of 2 mm. No liquefaction is caused.

Surface growth.
Gel. stab.



Gel. stab.

Potato.—Only a widespread moisture is seen at first, which by the third day has become like a thin layer of honey in appearance. It is moist and shining and very thin. After a time it becomes slightly darker.

Bouillon.—Diffuse cloudiness in twenty-four hours. The growth settles to bottom after two weeks, leaving the liquid tolerably clear. The bouillon acquires a faint greenish color.

Rosolic acid.—Becomes very slightly darker after three or four days.

Litmus milk.—Becomes darker blue on third day, but soon begins to lose its color. There is no coagulation. Reaction alkaline.

Sugar gelatin, deep stab.—Growth some distance down puncture, and on the surface similar to plain gelatin. No gas produced.

Indol.—Reaction negative.

Relation to temperature.—More rapid and abundant at 35° to 36° C.

Note.—In the Zeitschrift für Hygiene, Vol. IV, page 25, is an article on the "*Xerosis Bacillus* and its spore formation." The drawings correspond exactly with the organism just described, but the description, as far as it goes, is different.

Bacillus Vacuolatus.

(Fig. 27, Pl. III.)

Found at the depth of 7 feet in made soil, which had been paved for several years.

Character.—Strict aërobe; no development in an atmosphere of hydrogen.

Morphology.—Resembles the Klebs-Löffler bacillus very closely in its manner of staining, though it is much longer and more regular in shape. The rods are single, straight, and have rounded ends. Potato cultures form chains of several elements. The transverse bands which remain unstained give the rods a ringed appearance. It is best seen when the culture is about a week old.

Spores are large and oval, and may be seen at the end of twenty-four hours.

Motility.—Shows movements which are not very active.

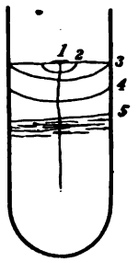
Flagella not demonstrated.

Colonies on gelatin plates.—Colonies appear in thirty-six to forty hours. Those on the surface grow rapidly, and in forty-eight hours have become one-half of a millimeter in diameter. They have a circular outline and are whitish, looking like drops of thin milk. By transmitted light they are bluish. The deep colonies are punctiform. × 80 . Deep are yellowish, finely granular disks, with even edges. Surface are yellowish in color, have an irregular, though clean-cut outline, and are granular or mottled in appearance. A nucleus, usually eccentric, is present in most of them.

Liquefaction takes place in saucer shape; not extensive. The colonies become more dense, and after five days a finely patterned edge with leafy outlines is seen around them. The mottled or marbled appearance is lost, and dark wavy lines running a long distance are seen. After this time the liquefaction is too extensive to make further observation.

Agar slant.—A thin translucent layer, almost without color, with irregular margins, is formed, and reaches the tube wall near the bottom after several days. It is smooth and shining, a faint greenish tint by transmitted light. The smooth surface is lost after some time by the growth of small rounded elevations, like colonies. The agar acquires a faint greenish tint.

Gelatin stab.—Good growth deep down, with a button on the surface. By the third day a small saucer of liquefaction has formed, which soon reaches the tube wall. At the end of a week the liquefaction is 1 cm. deep and the floor almost level, and covered with a whitish flocculent precipitate, the liquefied gelatin being clear. A mycoderma floats on the surface. The gelatin below the floor acquires a hazy look and liquefaction progresses slowly, being complete in about six weeks.



Gel. stab.

Potato.—Growth is seen on the second day along the line of inoculation. It soon becomes a slimy-looking, dirty light-brown layer, which covers most of the plug at the end of a week, passing around behind it. It is thick and moist and has a smooth surface. The color changes to yellowish brown as it grows older.

Bouillon.—Diffuse cloudiness by the second day. A thin pellicle which sinks easily is formed on the surface after ten days. The growth settles to the bottom, leaving the liquid quite clear.

Rosolic acid.—Becomes a cherry red in twelve to fourteen days, and a pellicle forms on the surface.

Litmus milk.—Becomes more blue after five or six days, then becomes watery and translucent. The color is almost all gone in twelve days, while a blue zone forms at the surface on the tube wall. The casein becomes dissolved without previous coagulation. Reaction alkaline.

Sugar gelatin, deep stab.—Grows only near surface. Same appearance as in plain gelatin.

Indol.—Good reaction with both sulphuric acid and sodium nitrite.

Relation to temperature.—More rapid and abundant at 35° to 36° C.

Bacillus Pinnatus.

(Figs. 28 and 29, Pl. III.)

Found at the depth of 6 feet in made soil, which had been paved for several years.

Character.—Grows well in an atmosphere of hydrogen.

Morphology.—Slender, short, straight rods with rounded ends, three to five times as long as broad. Occurs singly or in chains of two and three elements. In old cultures the rods are so short as to be oval.

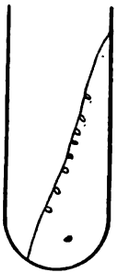
Spores have not been observed.

Motility.—The movements are slow and mostly rotatory, but now and then a rod will move some distance across the field.

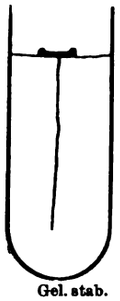
Flagella not demonstrated.

Colonies on gelatin plates.—Colonies become visible in thirty to thirty-six hours as bluish dots, which appear as finely granular disks with smooth edges under a low power. At the end of sixty hours the surface colonies are one-half of a millimeter in diameter, and stand up from the surface like minute drops of milk. The deep colonies are punctiform. × 80. Deep are finely granular, yellowish, and have smooth edges. The surface colonies are made up of a central portion of yellowish-brown color, finely granular, and growing uniformly lighter from center to edge. Outside of this is a zone of pale gray in which no structure can be made out. The division between the two is sharply defined by a line of light. The edges are clear-cut and even. Some colonies are oval, but most of them are circular, and all have the same general structure. The colonies grow more dense as they get older, the center becomes brownish, and the line of demarkation between the zones less distinct. The colonies never grow larger than 1 mm. in diameter, and no liquefaction is caused.

Agar slant.—A whitish translucent band forms along the line of inoculation, and by the third day is 3 mm. wide. It is very thin, moist, and has uneven edges. It seems to be watery, and flows down to the bottom, having a shiny look. It is not tenacious, and but little clings to the needle. About the tenth day whitish masses are seen, much more dense than the growth. On holding the tube to the light these are seen to be projections into the agar. They are oval in shape and extend about 1 mm. down. The surface of the agar has a roughened feel to the needle. Quite a quantity of the growth collects in the water at the bottom, and is milk white in color.


Agar slant.

Gelatin stab.—Growth occurs all along the line of puncture, and a button forms on the surface. In three days this button is one-fourth of a millimeter in height and 2 mm. in diameter, and is a porcelain white, smooth and glistening. It does not spread further, but the edges become elevated, forming a shallow basin. The gelatin after a time becomes cloudy on the surface, though no growth can be detected. No liquefaction occurs.


Gel. stab.

Potato.—Only a moisture along line of inoculation is seen at first. It soon spreads in a very thin, colorless layer over much of the surface. After four or five days it becomes a light, dirty brown, smooth, moist, and shining.

Bouillon.—Growth occurs first at bottom of the tube, but by the third day the liquid is diffusely cloudy. Dirty-looking whitish flakes form on the surface, but do not form a pellicle, and sink to the bottom on shaking the tube. The bulk of the growth settles to the bottom as a white mass.

Rosolic acid.—Becomes much lighter by the fourth day, and is completely decolorized in two weeks. The reaction is alkaline.

Litmus milk.—Shows no change until a week has passed, when it becomes darker, and finally changes to an indigo blue, almost. There is no coagulation and no further change seen at end of six weeks.

Sugar gelatin, deep stab.—Growth quite deep down puncture and on the surface. No liquefaction and no gas production.

Gelatin slant.—A porcelain white line 2 mm. wide is formed, which has even, elevated edges and a smooth, glossy surface. The whole surface of the gelatin becomes a cloudy white after some days, but no growth can be detected.

Indol.—Good reaction takes place with both sulphuric acid and sodium nitrite.

Relation to temperature.—Grows well at room temperature, but somewhat faster at 35° to 36° C.

Bacillus Diffusus (Frankland).

Found at the depth of 6 feet in made soil, which had been paved for several years.

Character.—Shows very slight growth in an atmosphere of hydrogen.

Morphology.—Slender straight rods, from five to seven times as long as broad. Occurs singly mostly, and in short chains.

Motility.—Some movement of an oscillatory character, but no progression.

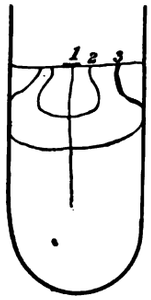
Flagella not demonstrated.

Spore formation not observed.

Colonies on gelatin plates.—Colonies do not become visible until the third day, when they appear as whitish dots. × 80. The deep colonies resemble those of the *Comma bacillus*, being coarsely granular with irregular margins. Near the edge wavy lines can be made out. The surface colonies have very little color at this stage, being faint bluish green. × 80. They show an indistinct nucleus, are irregularly circular, and have granular contents. As the colonies get older the deep ones reach the surface, and their margins become less distinct, a fringe-like border growing out over the surface of the gelatin. This border has leafy or deeply dentate margins, and is finely granular in structure, while the original colony is darker and very coarsely granular. Liquefaction takes place rather slowly, and each colony is in a saucer-shaped depression.

Agar slant.—A thin greenish-white layer is developed, which has smooth edges, and is moist and shining. As it increases in thickness it becomes a very pale-yellow color.

Gelatin stab.—Growth occurs along the puncture for some distance down, while a thin yellowish layer forms on the surface. This becomes concave after a few days and slowly sinks, liquefaction progressing slowly. Liquefaction causes a globe-shaped cavity, the tube wall being reached below the surface, leaving a ring of gelatin attached to the tube at the surface. On the liquid gelatin the original surface growth floats. After three weeks the floor becomes level, and has a slight whitish deposit on it. The process seems to stop here, further liquefaction not being observed. The liquefied gelatin is only slightly turbid.



Gel. stab.

Potato.—A greenish-yellow layer covers much of the surface in five or six days. It is quite thin, smooth, moist, and shining, and later acquires a transparent look and becomes more yellow.

Bouillon.—Diffuse cloudiness, not very intense. No pellicle forms on surface.

Rosolic acid.—Becomes slightly darker after four or five days.

Litmus milk.—Becomes a purer blue at end of six days, but the color is discharged soon after, being gone at end of the next week. No coagulation occurs. Reaction alkaline.

Sugar gelatin, deep stab.—Growth quite deep with button on surface, which slowly sinks. No gas production.

Indol.—Reaction negative.

Relation to temperature.—Grows more rapidly at 35° to 36° C.

Note.—This bacillus was first described by Grace and Percy Frankland in the Zeitschrift für Hygiene, Vol. VI, page 396. They give its growth on only four culture media, however, and its action on nitrates. The organism described above corresponds with their description quite closely, convincing me of their identity, though there are some slight differences.

Bacillus Gangliformis.

(Fig. 30, Pl. III.)

Found at a depth of 6 feet in made soil, which had been paved for a number of years.

Character.—Grows well in an atmosphere of hydrogen.

Morphology.—Large straight rods with rounded ends, five to seven times as long as broad. Forms chains of considerable length.

Motility.—Non motile.

Flagella not demonstrated.

Spores are large, oval, and formed in center of rod. They were found only in cultures on potato.

Colonies on gelatin plate.—Colonies are seen at the end of twelve hours. The deep resemble bits of asbestos fiber embedded in the gelatin. Those on the surface are circular saucers of liquefaction, with whitish masses in the center. × 80. The deep colonies are made up of coarse lines, irregularly disposed, and running from centers here and there, which are made up of more compact masses of lines. On the surface the colonies have a well-defined margin, which is bordered by a corona of fine spear points. In the liquefied gelatin are tangled masses of the thread-like lines, resembling the colonies of the potato bacillus. Here and there a bunch of lines will pass far out into the gelatin beyond the border. Liquefaction is rapid, and the deep colonies soon reach the surface, where they become like those which began there. At the end of twenty hours some of the colonies have attained a diameter of 6 mm. On the second day a thick mycoderma has formed on each colony. This is somewhat peculiar in its character. In the center is an island, then comes a space uncovered by growth, or very thinly covered; beyond this is a lace-work, the bars running in a radial direction. The edges are somewhat uneven.

Agar slant.—In twenty-four hours a bluish-white line 2 mm. wide has formed along the line of inoculation, spreading to the tube wall near the bottom. By the second day it has become twice as wide, is a dirty-white, dry-looking band, with irregular, ferny edges. It soon becomes thrown into large wrinkles, which have an elevation of 1 mm. The usual arrangement of the folds is a large one in the middle from top to bottom, with numerous smaller ones at right angles to this. The folding, however, is dependent on the vigor of growth and is different in each culture.

Gelatin stab.—By the end of twenty-four hours a saucer of liquefaction 3 mm. in diameter has formed, and growth is seen deep down the puncture. By next day the liquefaction has nearly reached the tube wall, but is shallow, and a pellicle has formed on the surface, which soon becomes wrinkled, and climbs up the tube wall 2 to 3 mm. The liquefaction becomes funnel shaped by the fourth day and by the tenth is almost complete. The growth settles to the bottom in whitish flakes, leaving the liquid clear, but few flocculi floating in it.

Potato.—A dry-looking white growth soon covers a large part of the surface, and by the third day is thrown into large folds. These soften down after some days, and at the end of two weeks the growth is a putty-colored, moist, slimy-looking layer. It is not tenacious, however, and does not cling to the needle.

Bouillon.—Becomes diffusely cloudy, a mycoderma forms on the surface in four or five days, and the bottom of the tube is covered with a dense whitish deposit, while cotton-like flocculi float in the liquid.

Rosolic acid.—No growth.

Litmus milk.—Reddish tint is seen about the third day, and coagulation in flocculi takes place in four to five days. The color is rapidly discharged and the casein is dissolved by the end of ten days. A white mass floats on the surface, and there is a whitish deposit at the bottom. The reaction is alkaline at end of two weeks.

Sugar gelatin, deep stab.—Good growth deep down, with rapid liquefaction. No gas produced.

Indol.—Faint color is produced by the addition of both sulphuric acid and sodium nitrite. It does not come at once.

Relation to temperature.—More rapid growth at 35° to 36° C.

Note.—This bacillus is much like the potato bacillus, and belongs to that group without doubt. The chief points of difference are its effect on milk, the appearance of the deep colonies, and the production of indol.

Bacillus Erodens.

(Fig. 31, Pl. III.)

Found at the depth of 6 feet in made soil, which had been paved for a number of years.

Character.—Requires oxygen for good development, though it shows some growth in an atmosphere of hydrogen.

Morphology.—Straight, thick rods, with rounded ends, from three to seven times as long as broad. Occurs singly for the most part.

Spores are formed rapidly at incubator temperature, the process being well advanced in sixteen hours. They are oval and formed near the center of the rods, often causing bulging of the wall of the cell. After a time the rods seem to shorten around the spore, and separated spores have not been demonstrated.

Motility.—Actively motile.

Flagella not demonstrated.

Colonies on gelatin plates.—Colonies are seen in twenty to twenty-four hours as minute whitish dots. ×80. Some resemble a small raspberry, while others show more of a nest-like arrangement, the granules not being so well marked. At the end of thirty-six hours the colonies are about one-tenth of a millimeter in diameter, and look white. ×80. They are round, with even edges, and dark gray in color. The distinctive feature about them is the movement of the contents, which is active and incessant. It is usually in the general direction of the hands of a watch, with many smaller eddies. On the third day the surface colonies may be 2 mm. in diameter, have well defined edges, and look as if they had been punched out. The even contour is usually kept at the surface, while the growth eats away the gelatin below. In some the action is uniform all around, so that the opening at the surface may be 2 mm. in diameter, while at the bottom it is 3 or 4 mm. The most constant feature, however, is the putting out of tunnels from the sides of the colony, some colonies having as many as five radiating from them. These extend 2 or 3 mm. and tend to curl up near the ends. A scant whitish growth is seen on the floor of each. The active movement in the colonies stops about the third day, probably from drying up, but is seen in the ends of these tunnels for five or six days. Occasionally a tunnel is sent

out from the deep colonies also. These tunnels with their curled ends are so striking and distinctive that they form an infallible guide to the diagnosis of this bacillus.

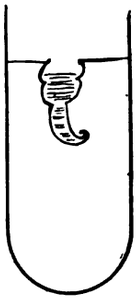
Agar slant.—Rapid growth takes place, and in twenty hours the surface is largely covered with a very thin, translucent, faintly greenish layer, which has leafy margins. After several days whitish specks, raised above the surface of the growth, appear, and the deposit in the water at the bottom takes on a faint salmon color.

Gelatin stab.—Growth occurs along the line of puncture, and by the third day a small funnel of liquefaction has formed at top. The deposit is very dense looking and almost white, resembling the pith of plants. After several days it begins to curl round at the bottom in the way the tunnels from the colonies do. The walls of the funnel often have an eroded look, and little tunnels are sent out into the gelatin, each one curling up at the end after a few days. The top of the funnel is usually circular, but sometimes is eaten out into a very irregular shape. The growth is very striking and characteristic.

Gel. stab.

Potato.—Growth not visible until third day, when it has spread widely over the surface in a very thin layer, moist and shining, about the color of honey. After some time it becomes dryer looking, and minute whitish dots appear in it.

Bouillon.—Becomes diffusely cloudy in twenty-four hours. After three weeks the growth has mostly settled to the bottom, leaving the liquid tolerably clear. No skin forms on the surface.


Gel. stab.

Rosolic acid.—Very slight growth. After five or six weeks the color is slightly deeper.

Litmus milk.—After six days becomes lighter but a purer blue. The color is discharged entirely by the twelfth day. Reaction decidedly alkaline. No coagulation of the casein is seen.

Sugar gelatin, deep stab.—Growth quite deep, with liquefaction. Same appearance as in plain gelatin. No gas production.

Indol.—Reaction negative.

Relation to temperature.—More rapid and abundant growth at 35° to 36° C.

Note.—In the report of the State board of health of Massachusetts for 1890, Jordan describes a bacillus resembling the above in many respects under the name "*Circulans.*" He mentions also having observed movement in the colonies of several other species, though not so marked or constant. The bacillus *Erodens* differs in its action on milk and in its way of boring into the gelatin. He speaks, though, of the funnel having a "ringed" appearance, which is seen in the *Erodens* also.

Bacillus Mesentericus Vulgatus.

Synonym: *Potato bacillus* (Flügge).

(Figs. 32, 33, and 34, Pl. III.)

Found at the depth of 6 feet in made soil, which had been paved for a number of years.

Character.—Strict aërobe; no development in atmosphere of hydrogen.

Morphology.—Rather slender rods, straight, with rounded ends, from five to seven times as long as broad. It occurs singly in short chains and in irregular groups, the rods lying side by side.

Spores are oval and formed near center of rod. At 35° to 36° C. spore formation is well advanced in sixteen hours. The spores are usually large in proportion to size of rod, many of them occupying two-thirds of the cell, and causing slight bulging of the walls.

Motility.—Feebly motile.

Flagella not demonstrated.

Colonies on gelatin plates.—Colonies appear within twenty hours as minute whitish dots. Those on the surface are almost without color, being faint bluish white. × 80. The deep colonies appear as gray or brownish dots with regular margins, while near the edges fine lines can be seen. The center is granular. On reaching the surface liquefaction goes on very rapidly, so

that in a few hours, if the weather be warm, the colony will attain a diameter of about 1 cm. Each colony is saucer shaped, the liquefied gelatin almost perfectly clear, being a faint grayish hue, and the edges smooth and regular, forming a perfect circle. × 80. Fine threads in tangled masses are seen in the liquefied gelatin resembling, especially near the center, "a ball of string," as Fränkel so aptly describes it. The edge of the colony is made up of a corona of fine spear points boring vertically into the gelatin, but somewhat wavy. These are much longer in a colony which has just reached the surface.

Agar slant.—An indistinct line along the inoculation soon appears, thin and spreading irregularly at the margins. By the third day the growth has covered almost the entire surface of agar, and the water at the bottom, and climbed up the tube 4 mm. from the surface of the water. It soon becomes thrown into fine wrinkles, becomes gray and then brownish, and looks as if fine powder had been sprinkled over it.

Gelatin stab.—Growth is mostly in upper part of stab, with a funnel of liquefaction at top. The liquefaction reaches the tube wall in thirty-six to forty hours, but is not very deep. At the end of ten days the floor has become level, about 1.5 cm. below the surface. It is covered with whitish flocculi, while the liquefied gelatin is almost clear. A thin skin may form on the surface, which becomes wrinkled.

Potato.—Growth occurs first along needle stroke, and looks much as if the potato had been scraped up from side to side. The surface of the plug is nearly covered on the third day, and the growth has passed entirely around it where moist. It is thrown into large folds, and looks as if dusted with fine powder. It soon becomes dry looking, and dark gray, and is piled up 2 mm. high. When touched with the needle it will draw out into quite long strings, being glutinous.

Bouillon.—Diffuse cloudiness on second day. A thin pellicle soon forms on surface. The growth settles to the bottom, leaving the liquid tolerably clear.

Rosolic acid.—No change observed.

Litmus milk.—Reddish tinge seen on third day, but no coagulation. The milk soon becomes watery and translucent from digestion of the casein, and the color is entirely discharged by the sixth day. A whitish mass floats on the surface, and in the bottom of the tube is a dense, viscous mass. The reaction is alkaline.

Sugar gelatin, deep stab.—Growth quite deep, with rapid liquefaction. No gas production.

Indol.—Reaction negative.

Relation to temperature.—Growth more rapid at 35° to 36° C., the entire surface of the agar being covered in twelve hours.

Bacillus Ginglymus.

(Fig. 35, Pl. III.)

Found at the depth of 1 foot in made soil, which had been paved for several years.

Character.—Very little growth in atmosphere of hydrogen.

Morphology.—Slender straight rods with rounded ends, from three to seven times as long as broad. Occurs singly and in short chains of two and three elements.

Spores are small, oval, and formed in center of rod.

Motility.—Peculiar in character. One end of a rod will appear to be fixed, and the rod moves on this as on a pivot. In others the center of rod acts as pivot on which the rod moves backward and forward. Some of the chains are motile also, the end rod waving from side to side, drawing the others after it, they showing no movement. It seems as though there was a hinge joint between them.

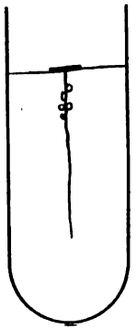
Flagella not demonstrated.

Colonies on gelatin plates.—Colonies seen in twenty to twenty-four hours as minute whitish dots. × 80. Gray granular disks with irregular edges. At end of thirty-six hours the surface colonies have reached a diameter of one-fourth of a millimeter, and are white. The deep are still punctiform. × 80. Surface colonies show central portion of orange-brown color, made up of finely interwoven lines, giving a somewhat wavy appearance. Around this is an outer zone of gray with well-defined edges, but showing no particular structure. The deep colonies

are yellowish granular disks. The colonies slowly increase in size, but seldom get larger than one-half of a millimeter in diameter. At end of a week a colorless, fringe-like border has formed around the edge of the surface colonies, which has a leafy outline and is finely veined. The center of the colonies becomes so dense that no structure can be seen. At this time each colony shows a dense center, next a wavy-looking zone of lighter brown which fades into a lighter colored and coarsely granular zone with a well-defined border, and lastly the colorless fringe. There is no liquefaction at end of ten days.

Agar slant.—A greenish-white line about 1 mm. wide forms along the line of inoculation in the course of thirty-six hours. It is slightly elevated, moist, and shining, and in a few days sends out a very thin, colorless layer from the edges, whose margins become very indistinct and almost impossible to locate positively.

Gelatin stab.—Growth along puncture occurs quite deep, and on the surface a grayish button is formed which attains a diameter of 2.5 mm., and has well-defined margins. After ten days indistinct outgrowths are seen along the puncture quite a distance below the surface. They are globular in shape. No liquefaction of the gelatin was seen at the end of twenty-eight days.


Gel. stab.

Gelatin slant.—A white line about 1 mm. is formed, not much elevated, smooth and shining, and with regular margins. No liquefaction took place, though at the end of a month the gelatin felt somewhat soft when touched with a needle.

Potato.—On second day a faint bluish discoloration is seen along line of inoculation. On third day the growth has spread over a large part of the surface of the plug in a thin yellowish layer, about the color of honey. It is moist and shining, and after two weeks becomes somewhat brown.

Bouillon.—Diffuse cloudiness is caused, the bulk of the growth finally settling to the bottom in gray masses.

Rosolic acid.—Becomes much lighter, so that at end of two weeks the color is entirely discharged.

Litmus milk.—Becomes a purer blue in twenty-four hours. No coagulation occurs. The milk gradually becomes translucent, and at end of two weeks is a beautiful violet color by transmitted light. This color fades slowly, but is still quite deep at end of six weeks.

Sugar gelatin, deep stab.—Growth quite deep along stab, with button on surface. No gas production.

Indol.—Reaction negative.

Relation to temperature.—Grows well at room temperature, more rapidly at 35° to 36° C.

Note.—This bacillus resembles the bacillus *Fissuratus* in many ways, and seems to be a variety of it. The chief differences are seen in the colonies on gelatin and in its non-liquefaction of gelatin. As the bacillus *Fissuratus* is a very slow liquefier, a hasty examination would make the two appear identical.

Bacillus Fissuratus.

(Figs. 36 and 37, Pl. III.)

Found at the depth of 1 foot in made earth, which had been paved for a number of years.

Character.—Shows very slight growth in atmosphere of hydrogen.

Morphology.—Small straight rods, two to three times as long as broad, with rounded ends Occurs singly for most part.

Spores are small, oval, and formed in center of rods.

Motility.—Only rotatory and oscillatory movements noted, which were active.

Flagella not demonstrated.

Colonies on gelatin plates.—Colonies may be seen at end of fourteen hours as minute whitish dots. × 80. No difference can be made out between the deep and superficial colonies at this stage. They are pale yellow, with irregular outline, darker at center, and give one the idea of flakes of mineral matter, or bits of shell. At the end of two days the surface colonies are 1 mm. in diameter and white, while the deep are still punctiform. × 80. Deep are orange brown and resemble a rosette somewhat. The surface colonies are circular in outline, but somewhat irregular.

Fig. 39.

Bac. Arborescens non-liquefaciens.
Colony 60 hrs. old. x 80.

Fig. 40.

Bac. Arborescens non-liquefaciens.
Colony 108 hrs. old. x 80.

Fig. 41.

Bac. Rodonatus.
A. Deep colony 36 hrs. old. x 80.

Fig. 42.

Bac. Rodonatus.
B. Surface colony 36 hrs. old. x 80.

Fig. 43.

Bac. Rodonatus.
Surface colony on 4th.day. x 80.

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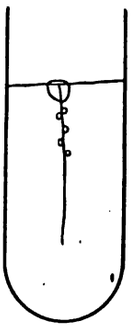
The center is brown in color, and appears broken and fissured in every direction. Next is a zone much darker, but also brown in color, and fissured even more than the center. It begins quite abruptly and fades toward the edge gradually until it becomes gray. The gelatin is slowly softened rather than liquefied, and the colonies attain a diameter of 2 to 3 mm. Around the white central portion can be seen a faint halo, which under the microscope shows the same fissured character as the body of the colony, but is without color. As the growth gets older the zones become less distinctly defined, while the fissures get plainer.

Agar slant.—Very thin layer, 3 to 4 mm. wide, with indistinct edges, which finally look so much like agar that it is impossible to tell just where the growth begins. It is greenish white and translucent.

Gelatin stab.—Slight growth down puncture, and on the surface a button forms. After eight or nine days this button has sunk into the gelatin, but no liquid can be detected, evaporation going on as rapidly as liquefaction. At some distance below the surface may be seen globular outgrowths ranged along the stab. They are white and indistinct. After two weeks the cup of liquefaction is only 6 mm. deep.


Gel stab.

Gelatin slant.—Band 4 mm. wide forms, which is not elevated, and has even edges. Surface of the growth is iridescent, and pale green by transmitted light. From the lower surface of growth rounded projections pierce the gelatin. The gelatin liquefies slowly and the growth slips to the bottom.

Potato.—Growth is scarcely visible until third day, though a bluish discoloration is seen along needle mark. It becomes abundant, of yellowish color, smooth, moist, and shining, resembling a thin layer of honey.

Bouillon.—Slight cloudiness at first. Growth settles to bottom later, leaving liquid tolerably clear.

Rosolic acid.—Color is almost entirely lost by the third day.

Litmus milk.—Becomes bluer without coagulation. After two weeks it becomes translucent, and is a beautiful violet color by transmitted light.

Sugar gelatin, deep stab.—Growth quite deep and on surface, same as plain. No gas production.

Indol.—Reaction negative.

Relation to temperature.—Grows more rapidly at 35° to 36° C.

Bacillus Arborescens Non-liquefaciens.

(Fig. 38, Pl. III, and Figs. 39 and 40, Pl. IV.)

Isolated from made earth at depth of 2 feet.

Character.—Strict aërobe. No growth in an atmosphere of hydrogen.

Morphology.—Straight, slender rods, with rounded ends, from seven to thirteen times as long as broad. Occurs singly and in chains of several elements.

Spores not demonstrated.

Motility.—Rods turn almost end for end, and back again. No progression noticed.

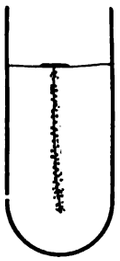
Flagella not demonstrated.

Colonies on gelatin plates.—Visible at end of forty-eight hours, as bluish, indistinct, cloudy dots, easily overlooked. The deep colonies can not be distinguished from those on surface. They resemble the colonies of the *Ramosus*, but are less distinct, smaller and not so white, nor do they grow so rapidly. Each colony consists of an axial trunk, both ends of which split up into numberless fine lines, somewhat interlaced, but on the whole radially disposed. These terminal branches are six to seven times as long as the trunk itself. In some colonies the trunk splits into several large branches, each of which gives rise to many fine ones, the whole giving a star-shaped figure. Others are made up of several sticks loosely laid together, each one of which splits up into fine branches at both ends; while others again are like the branch of a tree, one end breaking into smaller branches, which then break up into finer lines, while the other end is larger, and breaks up directly into the fine brush like filaments. After four days the colonies become more plainly visible, like bluish clouds. Under the low power (80 diameters) the fine terminal lines

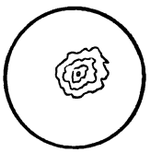
have increased very much, so that the entire mass assumes a round shape. In many there are two foci, as it were, formed by the ends of the trunk where the branching first began. The lines increase for some time, growing longer and becoming more tangled, making the colony more dense in appearance, but even after two weeks they look only like bluish, cottony masses, none more than 1 mm. in diameter, and to the naked eye showing the bi-polar arrangement. There is never any liquefaction perceptible.

Agar slant.—Growth is barely perceptible until the third day, when it is found to have spread quite widely over the surface. It resembles the growth of the bacillus of influenza very closely. Along the needle stroke is a faint colorless line, and on either side may be seen, by close inspection, colonies which resemble drops of moisture. They are 0.5 mm. in diameter, colorless, and always discrete. The water at bottom shows a whitish growth. After three weeks the growth becomes somewhat more opaque, but never takes on color, and is always hard to see. It is best seen by transmitted light.

Gelatin stab.—On second day a faint whitish growth is seen on surface and along the stab. The surface growth spreads until about the sixth day, when it is 3 mm. in diameter, and is irregular in outline and thickness, piling up at the center. It looks as though layer after layer had grown from the center on the surface of the one preceding, each being of irregular outline, and extending a lesser distance from the common center. It acquires a white metallic luster about the same time. Along the stab fine outgrowths appear about the fifth day, while deeper the colonies appear as separate, minute, white dots, which extend to bottom of puncture.



Gel. stab.



Gel. stab. Surface growth.

Potato.—No growth can be seen until the seventh day, when it acquires a somewhat different color from the potato, which later (sixteen days) becomes about the color of honey. Until the seventh day, only a moisture can be seen. It is a very thin layer, smooth, moist, and looks glazed.

Bouillon.—On third day a slight, diffuse cloudiness is seen, which increases somewhat, but never becomes dense. Later the growth settles to bottom, leaving liquid almost clear.

Rosolic acid.—Very slight growth and no change of color.

Litmus milk.—Color is discharged to some extent by the third day. About the tenth day a reddish tint is seen, and the casein is coagulated in flocculi. Reaction acid. There is no subsequent digestion of the casein up to five weeks.

Sugar gelatin, deep stab.—No growth seen for a week, when a single colony developed deep white and sending out fine branches.

Gelatin slant.—At first a faint whitish line, 1 mm. wide, along needle mark. Later this became an elevated white band, 2 mm. wide, with uneven surface and irregular edges. No liquefaction after three weeks.

Indol.—Negative.

Relation to temperature.—More rapid at 35° to 36° C.

Note.—In the Zeitschrift für Hygiene, Vol. VI, page 379, the Franklands first described an organism under the name bacillus *Arborescens*. The single colonies on gelatin of the bacillus here described correspond so closely to the description of the organism described by them, that I have retained the name so well applied, qualifying it by a striking distinctive characteristic—the non-liquefaction of gelatin.

Bacillus Rodonatus.

(Figs. 41, 42, and 43, Pl IV)

Found at the depth of 1 foot in made earth, which had been paved for a number of years.

Character.—Requires oxygen for its best development, though a fair growth occurs in an atmosphere of hydrogen.

Morphology.—Short rods with rounded ends, but little longer than broad, forming ovals. Resembles the bacillus *Prodigiosus* very closely. Occurs singly and in irregular groups; no chains formed.

Spore formation not observed.

Motility.—Non-motile.

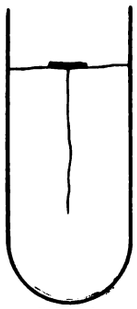
Flagella not demonstrated.

Colonies on gelatin plates.—Colonies seen in about thirty hours. The deep are punctiform and white, and under a low power (× 80) resembles masses of amorphous crystals heaped up. They are yellowish brown with very irregular edges. In the body of the colony the edges of the separate masses can be seen overlapping one another, the whole resembling a rosette. At the end of thirty-six hours the surface colonies are one-fourth of a millimeter in diameter, circular, and white. × 80. Appearance much the same as deep, the mass being more dense, and the central structure not so apparent. The center is yellowish brown and coarsely granular, while at points the outlines of the masses composing it can be seen. They became more clearly visible toward the edge, giving a petal-like arrangement. Beyond is an outer zone which is grayish and finely granular, and fine veins run through it. The colony is circular, with well-defined edges, which are notched here and there. The growth is rather slow, so that at the end of sixty hours the surface colonies are not quite 1 mm. in diameter. At end of eighty-four hours many of them are so dense that it is hard to make out any structure, but in some, by using strong light, the characteristic rosette form can be seen. The deep colonies grow slowly, the masses becoming larger and more regularly disposed, the resemblance to a rosette becoming more marked. In the surface colonies the petal-like arrangement at the edge becomes more distinct, the leaves growing longer and narrower in proportion. As the colony grows older it increases in thickness layer by layer, piling up in the center and growing thinner toward the edges, the color ranging from a dark reddish brown in the center to a pale yellowish gray at the edge. After a week the colonies acquire a fern-like border. There is no liquefaction.

Agar slant.

Agar slant.—A white translucent layer is formed which never spreads very widely. It is not much elevated and has leafy edges. After four or five days a very thin, colorless layer is seen around the edges of the first growth, which has ferny outlines.

Gelatin stab.—Same growth along line of puncture, but mainly on surface, where a thin white layer with irregular leafy edges is formed. It attains a diameter of 4 or 5 mm. No liquefaction at end of three weeks.


Gel. stab.

Gelatin slant.—Greenish-white band, 2 mm. wide, elevated, smooth and shining. The edges are irregular, and after some days become finely dentate.

Potato.—A yellowish layer, moist and shining, which finally covers most of the plug, is formed. At end of a week it becomes a dirty, brownish color, but no further change occurs.

Bouillon.—Diffuse cloudiness seen in twenty-four hours. After a few days a thin, friable pellicle forms on surface, while the growth collects at bottom in dense whitish masses.

Rosolic acid.—Loses color entirely in six or seven days.

Litmus milk.—Becomes a pure blue in three or four days. The color is then slowly discharged and is entirely lost at end of ten days. The milk becomes watery. No coagulation at any time.

Sugar gelatin, deep stab.—Growth in upper half of stab, with button on surface. No gas produced.

Indol.—Reaction negative.

Relation to temperature.—Grows more rapidly at 35° to 36° C.